AD_____

Award Number: DAMD17-02-1-0070

TITLE: Prevention of Post-Radiotherapy Failure in Prostate Cancer by Vitamin D

PRINCIPAL INVESTIGATOR: Srinivasan Vijayakumar, Ph.D.

CONTRACTING ORGANIZATION: The University of California
Davis, California 95616-8670

REPORT DATE: March 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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Prevention of Post-Radiotherapy Failure in Prostate Cancer by Vitamin D

5. FUNDING NUMBERS
DAMD17-02-1-0070

6. AUTHOR(S)
Srinivasan Vijayakumar, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of California
Davis, California 95616-8670

E-Mail: vijay@ucdavis.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
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13. ABSTRACT (Maximum 200 Words)

Prostate cancer patients receive either surgery or radiation therapy as treatment for cancer. Among patients receiving radiation therapy, nearly 50% have an elevation of PSA within five years of treatment. These patients then receive hormone treatment. In this study, we wish to test the theory that chemopreventive agents, which show the ability to prevent or delay the growth of prostate cancer cells in the laboratory, may also prevent or delay the reappearance of prostate cancer in patients who have undergone radiation to treat their prostate cancer. We propose to have prostate cancer patients who have already undergone radiation treatment take a non-toxic chemopreventive agent [a synthetic form of vitamin D, 1α-hydroxyvitamin D5] for two years and see if their reoccurrence rate can be decreased. Unlike regular vitamin D, D5 does not make calcium in the bloodstream reach levels that cause serious side effects. Forty patients will participate. They will be randomized to D5 or placebo arms. A biopsy will be done at the end of the study and the tissue will be analyzed for any benefit of D5 in decreasing the recurrence of prostate cancer and also for any differences between the groups in terms of expressed intermediate molecular biomarkers.

14. SUBJECT TERMS
Radiation therapy, vitamin D analog, PSA, biomarkers, D5, prostate cancer, chemoprevention

15. NUMBER OF PAGES
11

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

TABLE OF CONTENTS

INTRODUCTION

We plan to conduct a phase I/II safety/chemoprevention study to determine whether taking a non-toxic Vitamin D analog, 1α(OH)D5 (D5), can safely delay prostate cancer recurrence when administered after radiation therapy (RT). The newly synthesized analog 1α(OH)D5 (1α-Hydroxy-24-ethyl-cholecalciferol) has shown anti-tumor activity at non-hypercalcemic concentrations in animals. Based on our preliminary research, we believe D5 can be given in effective doses without causing harmful side effects. Forty randomized patients will receive either D5 or placebo, 12-60 months after completion of RT (20 patients/arm). During the study patients will be closely monitored for hypercalcemia as well as other potential toxicities. At the end of the study, subjects will receive final laboratory and clinical evaluations and undergo a prostate biopsy. Study endpoints include differences between study groups in drug tolerance and compliance, toxicity, quality of life, biomarker presence and proportion of patients developing PSA-based biochemical failure or clinical failure. Biopsies will be evaluated for selective markers indicating any benefit of D5 in decreasing the recurrence of prostate cancer and also for any differences between the groups in terms of expressed intermediate molecular biomarkers. Patients will continue to be followed for any clinical recurrences or toxicity as part of their usual cancer care.

BODY

The following are the tasks for this study:

Task		Progress
Task 1	Obtain necessary clinical trial approvals.	In progress
Task 2	Register patients to start the clinical study.	Not yet initiated
Task 3	Following up patients on study.	Not yet initiated
Task 4	Complete the clinical study.	Not yet initiated
Task 5	Follow up patients with Vitamin D treatments.	Not yet initiated

With regard to Task 1, during the past year we have:

Date	Progress
October 26, 2004	Updated our Statement of Work (SOW) (Appendix 1).
November 4, 2004	Since the process of required approvals is taking longer than expected, we requested and received a no-cost extension from the DOD for the study, to February 2006 (Appendix 2).
December 6, 2004	Obtained DOD approval for the study (Appendix 3).
December 15, 2004	Obtained UC Davis IRB re-approval for the study, accepting the DOD's changes (Appendix 4).
February 22, 2005	Requested annual renewal of this study with our IRB (Appendix 5).

We await FDA approval for the study drug, which we believe will happen soon. We are presently conducting stability tests on the pill (see Appendix 6 for e-mail correspondence regarding the status of FDA approval).

KEY RESEARCH ACCOMPLISHMENTS

As this is a clinical study, only key findings generated from this clinical study can be considered as key research accomplishments. Since the clinical trial has not even begun and is pending approval by the FDA, we have no research accomplishments at this time.

REPORTABLE OUTCOMES

We have no reportable outcomes yet. However, during the past year we published one paper about this project (see "References") and have another one in progress.

CONCLUSIONS

We have not initiated the research on this project. We await FDA approval for the study drug.

REFERENCES

Please see Appendix 7 for a copy of the following paper, regarding this study, and published during the past year:

Packianathan S, Mehta RG, Mehta RR, Hall WH, Boerner PS, Beckett LA, Vijayakumar S. Designing a randomized phase I/II prostate cancer chemoprevention trial using 1alpha-hydroxy-24-ethyl-cholecalciferol, an analogue of vitamin D3. Cancer J. 2004;10(6):357-67.

A copy of the updated version of the protocol is submitted as Appendix 8.

APPENDICES

1. Revised Statement of Work (SOW), dated October 26, 2004
2. No-cost extension approved by the DOD
3. DOD Letter of Approval
4. UC Davis IRB Letter of Approval
5. Annual renewal request for this study with our IRB
6. E-mail correspondence regarding FDA approval for the study drug
7. Article by Packianathan *et al.*, regarding this study.
8. Protocol approved by DOD and IRB

Appendix 1

Revised Statement of Work
October 26, 2004

Protocol, *"A Phase I/II Double-Blinded, Randomized Clinical Trial to Prevent/Delay Biochemical and Clinical Failure in High-Risk, Non-Metastatic Prostate Cancer Patients After Radiotherapy, Using 1α-Hydroxyvitamin D5 Versus Placebo: A Tolerance-Finding and Intermediate Biomarker Response-Seeking Study"*, Submitted by Srinivasan Vijayakumar, M.D., University of California, Davis, Sacramento, California, Proposal Log Number PC010148, Award Number DAMD17-02-1-0070, HSRRB Log No. A-11241

The original "Statement of Work" envisioned three years of studies, including basic scientific research, at the University of Illinois at Chicago, the PI's original institution. As the basic scientific research was deleted from the project per the DOD's scientific review panel, and as the PI has moved to the University of California, Davis, a revised "Statement of Work" is necessary.

The proposed revised "Statement of Work," detailed below, describes a clinical trial that will be completed in three years. As extensions are generally granted one year at a time, we will be requesting additional extensions to complete the entire clinical trial.

Year 1 (after attaining final FDA, DOD and IRB approvals)

1st month:	Begin recruiting and registering patients
4th month:	Conduct one-month run-in period with study subjects
5th month:	Patients begin receiving either placebo or study medication for 2-year period of clinical trial.
6-12 months:	Continue conducting clinical trial with study subjects.

To be requested in subsequent extensions:

Year 2
Continue conducting 2-year clinical trial with study subjects.

Year 3

1-4 months:	Continue conducting clinical trial with study subjects.
5th month:	Perform end-of-study biopsies; determine selected markers in biopsies.
5-12 months:	Analyze study specimens for biomarkers and analyze study data; prepare manuscripts and project report; evaluate effects of vitamin D5 treatment on patients.

Years 4-6
Follow-up of patients continues at no cost to DOD.

Appendix 2

ASSISTANCE AGREEMENT

S. Vijay

AWARD TYPE: [✓] GRANT (31 USC 6304) [ ] COOPERATIVE AGREEMENT (31 USC 6305) [ ] OTHER TRANSACTION (10 USC 2371)

AWARD NO: DAMD17-02-1-0070 Modification P00002	EFFECTIVE DATE See Grants Officer Signature Date Below	AWARD AMOUNT $545,211.00	Page 1 of 1 Rita E. Johnson 301-619-2359 301-619-2505 (FAX)

PROJECT TITLE: Prevention of Post-Radiotherapy Failure in Prostate Cancer by Vitamin D

CFDA 12.420

PERFORMANCE PERIOD: 1 Mar 02 - 31 Mar 06 (Research ends 28 Feb 06)	**PRINCIPAL INVESTIGATOR:** Srinivasan Vijayakumar, Ph.D.
AWARDED AND ADMINISTERED BY: U.S. Army Medical Research Acquisition Activity ATTN: MCMR-AAA-B 820 Chandler St. Fort Detrick Maryland 21702-5014	**PAYMENTS WILL BE MADE BY:** EFT:T Army Vendor Pay DFAS-SA/FPA (888) 478-5636 500 McCullough Avenue San Antonio, TX 78215
DUNS No: 047120084 **TIN No:**	*(SEE PARAGRAPH TITLED "PAYMENTS" FOR INSTRUCTIONS)*
AWARDED TO: Regents of the University of California Office of the Vice Chancellor for Research, Sponsored Programs, 118 Everson Hall, One Shields Avenue, University of CA Davis, CA 95616-8670	**REMIT PAYMENT TO:** Regents of the University of CA Cashier's Office 1200 Dutton Hall, One Shields Ave University of CA Davis, CA 95616-8549

ACCOUNTING AND APPROPRIATION DATA: N/A

010637 NCT

SCOPE OF WORK:

The purpose of this modification is to

1. Extend the period of performance:

FROM: 1 Mar 02 - 31 Mar 05 (research ends 28 Feb 05)

TO: 1 Mar 02 - 31 Mar 06 (research ends 28 Feb 06)

2. Incorporate by reference the revised SOW dated 26 Oct 04.

All other terms and conditions remain unchanged.

Reviewed & Processed
OVCR
Sponsored Programs
Date 11/19/04
Initial WC
Copies to:
[x] Dept: Med Rad. Onc
[x] Extm Acctg.
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[ ] Equip. Inv.
[ ] Int. Med. Fin.
[ ] Engr. Dean
[x] Med. Dean
[ ] VM Dean
[ ] Other PI

RECIPIENT		GRANTS OFFICER	
ACCEPTED BY: No signature required. See email dated 10/29/04. SIGNATURE		UNITED STATES OF AMERICA Joseph S. Little SIGNATURE	
NAME AND TITLE	DATE	NAME AND TITLE JOSEPH S. LITTLE GRANTS OFFICER	DATE 4 Nov 04

USAMRAA FORM 60-R, Feb 00

Srinivasan Vijayakumar/PHY/HS/UCD
12/06/2004 09:31 AM

To: Philip Boerner/SOM/HS/UCD@UCDavis
cc:
bcc:
Subject: Fw: Your revisions for A-11241

—— Forwarded by Srinivasan Vijayakumar/PHY/HS/UCD on 12/06/2004 09:30 AM ——

"Ferrandino, Donna Dr AMDEX" <donna.ferrandino@us.army.mil>
12/06/2004 09:26 AM

To: <vijay@ucdavis.edu>
cc: "Mishra, Nrusingha C Dr USAMRMC" <nrusingha.mishra@us.army.mil>
Subject: Your revisions for A-11241

SUBJECT: Protocol, "A Phase I/II Double-Blinded, Randomized Clinical Trial to Prevent/Delay Biochemical and Clinical Failure in High-Risk, Non-Metastatic Prostate Cancer Patients After Radiotherapy, Using 1α-Hydroxyvitamin D5 Versus Placebo: A Tolerance-Finding and Intermediate Biomarker Response-Seeking Study," Submitted by Srinivasan Vijayakumar, M.D., University of California, Davis, Sacramento, California, Proposal Log Number PC010148, Award Number DAMD17-02-1-0070, HSRRB Log Number A-11241

Dear Dr. Vijayakumar:

We have completed our review of your revisions that were sent in response to the recommendations made at the HSRRB meeting on 28 July 2004. At this point, you are authorized by the Vice Acting Chair of the HSRRB to return to your local IRB at USC, and seek its approval of the revised protocol and supporting documents.

Please provide us with copies of the letter of approval from your IRB and of the latest revised documents that it approves. After receipt of these documents, the Vice Acting Chair will issue the approval of your protocol to the Army contract office, who will issue the official approval of your protocol to your institution's grants office. Please be reminded that no work with human subjects may begin on your study until the official approval notification is issued.

There is one outstanding issue regarding your protocol: for an IND study, the PI and the co-investigators must have GCP training in addition to Human Subjects Protection training. (This regulation covers just those who are considered to be co-investigators, not every member of the

research team.) If you have documentation of GCP training available, could you please send it to us for the file? Also, please send us a copy of the SOP for the study drug manufacturing when that becomes available.

Thank you for your cooperation and hard work during this process. I look forward to receiving your final paperwork for this protocol.

Sincerely,
Donna

Donna S. Ferrandino, PhD
Human Subjects Protection Scientist (AMDEX Corp)
U.S. Army Medical Research and Materiel Command
Office of Research Protections
504 Scott Street
Ft. Detrick, MD 21702
(301)619-6237 (tel)
(301)619-7803 (fax)
donna.ferrandino@det.amedd.army.mil

Appendix 4

UNIVERSITY OF CALIFORNIA, DAVIS
OFFICE OF THE VICE CHANCELLOR FOR RESEARCH
HUMAN SUBJECTS REVIEW COMMITTEES

COPY

REQUEST FOR MODIFICATION/AMENDMENT

SECTIONS I AND II TO BE COMPLETED BY THE PRINCIPAL INVESTIGATOR

Section I **Today's Date: December 7, 2004**

PI Name: Srinivasan Vijayakumar, M.D. Department: MED RADIATION ONCOLOGY (049063)

Contact: Phil Boerner 4-3981 (ph) fax 454-4614

Telephone: (916) 734-7888 Fax No. (916) 734-8011

Protocol No.: 200412214-1 Sponsor: Department of Defense (Z1000959)

Title of the Study: A Phase I/II Double-Blind, Randomized Clinical Trial to Prevent/Delay Biochemical and Clinical Failure in High-Risk, Non-Metastatic Prostate Cancer Patients After Radiotherapy, Using 1a-Hydroxyvitamin D5 Versus Placebo: A Tolerance-Finding and Intermediate Biomarker Response-Seeking Study

Section II

Please summarize your request for modification/amendment below ("see attached" is not acceptable). You must also attach all supporting documentation, i.e., revised consent form, revised description of study, sponsor's revised protocol, etc. Attach additional page if more space is needed.

After the UCD IRB approved this study on 4/28/04, the sponsor's Human Subjects Research Review Board (HSRRB) reviewed the study and requested minor changes to the protocol and the consent form, before granting their approval on 12/6/04. We have attached our letter responding to the HSRRB, which details these changes. We have also attached revised copies of the protocol and the consent form. The changes were:

1. We clarified the ingredients of the placebo and the study drug (page 12 of the protocol; item a.4 in the letter).
2. We confirmed that the tables describing the lab tests, etc. that study subjects will undergo are consistent throughout both documents (no changes were made) (item a.5/letter).
3. We reformatted the documents to have consistent notation for the daily study drug dose (item a.6 in letter).
4. We clarified that the study drug is not a CTEP drug, and substituted the MEDWATCH reporting information and form for the CTEP information and form (item a.7/letter, page 33/protocol).
5. We provided a description of the procedure for maintaining treatment randomization codes and procedures for breaking the codes (item b.3/letter, page 35/protocol).
6. We provided a description of the packaging and labeling of the study drug (item b.4/letter, page 12/protocol).
7. We provided a description of the controls and methods that will be used to minimize bias on the part of the subjects, investigators, and analysts (item b.5/letter, page 21/protocol).
8. We added a statement that representatives of the DOD (the study sponsor) may inspect the research records (item b.6/letter, page 19/protocol).
9. We added a description of the procedures regarding the collection, labeling, storage, use, and disposal of blood and urine samples for all study subjects (item b.7/letter, page 16/protocol).
10. We modified the protocol and consent form to reflect that urine samples will be taken from subjects (we had mentioned that there would be tests for urine electrolytes) (item b.10/letter, page 16/protocol, page 11/consent form).
11. We have deleted a sentence in the protocol that said that patients would receive a copy of the protocol; this was in accordance with the earlier request by the UCD IRB to do this (item b.11/letter).
12. We deleted Appendix IX, the "Vitamin D Patient Handout," again in accordance with the earlier request by the UCD IRB (item b.12/letter).

13. We have substituted the DOD's most recent language, under submission of AEs, regarding reporting of adverse events to the study sponsor (item b.13/letter, page 34/protocol).
14. We added an abbreviated list of the study's inclusion/exclusion criteria to the consent form (item d.1/letter, page 9/consent form).
15. We added a statement that study subjects are not to take Vitamin D supplements during the study, and that they should inform the study investigators if they are taking any multi-vitamins (item d.3/letter, page 8/consent form).
16. We added spaces for the permanent addresses of study subjects at the end of the consent form (item d.4/letter, page 22/consent form).
17. We modified Appendices V, VI, and XIII (QOL survey, symptom scale, and pill diary) to include the title of the study. We also renumbered Appendix XIII and Appendix VIII.
18. We eliminated a bit of duplicate information throughout the protocol. Specifically:
a. The table that covers the symptoms of hypercalcemia that appeared on pages 32 and 34 is no longer on page 34.
b. The same paragraph describing the informed consent process that appeared on both pages 28 and 29 has been deleted from page 28.
c. We have eliminated the first instance of the same paragraph about the medical monitor that appeared on pages 18 and 36.
d. The first paragraph on the run-in period on page 19 has been taken out, and the one on page 36 kept in.
19. We have changed the medical monitor for the study from Dr. Rachel Chou, who left the university in July 2004, to Dr. Allan Chen.
20. One of the study coordinators, Cheri Koppe, recently got married and changed her name to Cheri Grelle, and so her name has been changed throughout the protocol and consent form.

That summarizes the changes to the study protocol and consent form. We still await final approval from the FDA for the study drug and so, even with UCD IRB approval, we will not start the study until we have FDA approval for D5.

SECTION III TO BE COMPLETED BY THE HSRC OFFICE

Section III

Modification/Amendment Approval

The signature below acknowledges review and approval by the Human Subjects Review Committee for the modification/amendment indicated above. Supporting documents are attached.

Signature of Approval

John Anderson, MD
Chair, IRB
University of California, Davis

DEC 15 2004
Date of approval

MODIFICATION APPROVED by the Institutional Review Board at the University of California, Davis on:
Approval Date: 12-15-04
Expires On: 4-28-05

Required Copies: original plus one copy of this form plus two copies of all supporting documentation. Please highlight or use bold font to indicate where changes/additions occur .
Submit to: Human Subjects Review Committees, Ambulatory Care Center, Suite 3870, UCDMC

February 22, 2005

TO: **Chair**
Office of Human Research Protection

FROM: **Srinivasan Vijayakumar, M.D.**
Principal Investigator
Department Chair
Radiation Oncology

RE: **HSPN 200412214-1: UCDCC#141: A Phase I/II Double-Blind, Randomized Clinical Trial To Prevent/Delay Biochemical and Clinical Failure In High-Risk, Non-metastatic Prostate Cancer Patients After Radiotherapy, Using 1a-Hydroxyvitamin D5 Versus Placebo: A Tolerance-Finding and Intermediate Biomarker Response Seeking Study**

PROGRESS NOTES:

Please find enclosed 25 copies of this memo, renewal notice, approved description of study, approved consent form, and approved modification for committee review. We have also attached one copy of the currently approved protocol.

1. There are no results to date regarding this protocol. We are still waiting for FDA approval for the study agent, therefore, no patients have been enrolled to this study.
2. We plan to keep this study open during the coming year, anticipating 10-15 patients on this study in the coming year, once we are able to accrue patients.
3. There have been no problems in the past year.
4. There is one change to the study, which is listed in the approved modification attached.
5. We initially planned to enroll 40 patients to this study in total.
6. There are no subjects enrolled to this study to date.
7. There were no subjects who were offered this study who then declined to participate.
8. N/A.
9. N/A.
10. N/A.
11. N/A.
12. Please see attached renewal notice with appropriate signatures.
13. Please see the attached consent form.
14. Please see the attached description of study.
15. There have been no adverse events since the study opened.
16. There has been one modification approved since the study opened, which is attached.
17. Please see attached two copies of the grant.
18. There have been no findings thus far regarding this study.

UCDCC#141 (Page 2 of 2):

19. N/A.
20. This study has not been audited in the past year.

Thank you,
Enclosures

Appendix 6

Mehta Rajendra <rmehta@iitri.org>

03/16/2005 08:59 AM

To:
"'Ferrandino, Donna Dr AMDEX'" <donna.ferrandino@us.army.mil>, vijay@ucdavis.edu

cc:
"Mishra, Nrusingha C Dr USAMRMC" <nrusingha.mishra@us.army.mil>, Mehta Rajendra <rmehta@iitri.org>

Subject:
RE: D5 Clinical Trial IND: dose escalation and stablity studies Number DAMD17-02-1-0070 A-11241

Dear Dr. Ferrandino:

Thank you for your mail today. As I mentioned in my previous e-mail in February, we completed a stability study for the D5 under GLP guidelines and the product (analog in the pill) is very stable. No degradation was noticed at room temperature for 10 days.

We submitted these data to our consultant [Ms. Trag] at the Midwest Consulting Services, South Bend, IN on February 25, 05

According to Ms. Trag, we also need to show 'chemical stability' for D5. We already had submitted the chemical stability information to the FDA showing that the D5 is very stable but this needs to be demonstrated under the identical LC-MS condition used for evaluating the stability of the product (D5 in the pill), according to Ms. Trag.

So that experiment is in progress. Once we have that data available to us within the next week or so, we will submit the data to FDA (through Ms. Trag) and hopefully the approval will be given to us soon there after.

I will keep you informed regarding the progress and the correspondence from the FDA.

Thank you very much, with regards,

Sincerely,
Raju Mehta

Rajendra G. Mehta, PhD
Assistant Vice President and Head
Carcinogenesis and Chemoprevention Division
IIT Research Institute
Professor, Biological Sciences, IIT
10 West 35th Street
Chicago, IL 60616
Phone: (312) 567-4970
Fax: (312) 567-4931
e-mail: RMehta@iitri.org

Appendix 6

-----Original Message-----
From: Ferrandino, Donna Dr AMDEX [mailto:donna.ferrandino@us.army.mil]
Sent: Tuesday, March 15, 2005 1:18 PM
To: Mehta Rajendra; vijay@ucdavis.edu
Cc: Mishra, Nrusingha C Dr USAMRMC
Subject: RE: D5 Clinical Trial IND: dose escalation and stablity studies Number DAMD17-02-1-0070 A-11241

Dear Dr. Vijayakumar and Dr. Mehta:

Could you update us on the status of the FDA approval for your study for the use of D5? The last email we received was on 8 February.

Thank you,
Donna

Donna S. Ferrandino, PhD

Human Subjects Protection Scientist (AMDEX Corp)

U.S. Army Medical Research and Materiel Command

Office of Research Protections

504 Scott Street

Ft. Detrick, MD 21702

(301)619-6237 (tel)

(301)619-7803 (fax)

donna.ferrandino@det.amedd.army.mil

Appendix 6

From: Mehta Rajendra [mailto:rmehta@iitri.org]
Sent: Tuesday, February 08, 2005 4:44 PM
To: Mishra, Nrusingha C Dr USAMRMC
Cc: srinivasan.vijayakumar@ucdmc.ucdavis.edu; Ferrandino, Donna Dr AMDEX
Subject: RE: D5 Clinical Trial IND: dose escalation and stablity studies Number DAMD17-02-1-0070 A-11241

Dear Dr. Mishra:

It was very nice talking to you earlier today. As you know we had submitted the original application for IND to FDA in 1998. At that time we wanted to know what would be the requirements for appropriate submission of the FDA application. Since hen we collected a large body of data including a preclinical toxicity in rats and dogs under GLP guide lines, the IRB approved protocol, toxicity parameters, GMP synthesis of 1a(OH)D5, formulation of the compound and stability studies. Completion of these studies took a long time. Since then we submitted the application to FDA for the approval twice. The first time there was some problem with the protocol, which we fixed and then the second time they did not like the stability studies. This time, they want us to conduct the stability studies using two methods such as HPLC and LC-MS.

Since I moved to IIT Research Institute since then, we gave a contract to a professional pharmacology laboratory at IITRI. The studies are complete now using both HPLC and LC-MS procedures. The studies are being evaluated by the Quality assurance group and we should be able to get it within the next week or so. Once we have that we will be able to submit it once more to FDA. This time we are confident it should be approved for Phase I/II clinical trials to be conducted at UIC by Dr. Das Gupta.

That is where we stand. If you need any other information, please feel free to contact me. With warm regards,

Sincerely,
Raju Mehta

Rajendra G. Mehta, PhD
Assistant Vice President and Head
Carcinogenesis and Chemoprevention Division
IIT Research Institute
Professor, Biological Sciences, IIT
10 West 35th Street
Chicago, IL 60616

Phone: (312) 567-4970
Fax: (312) 567-4931
e-mail: RMehta@iitri.org

Appendix 7

Designing a Randomized Phase I/II Prostate Cancer Chemoprevention Trial Using 1α-Hydroxy-24-Ethyl-Cholecalciferol, an Analogue of Vitamin D_3

S. Packianathan, MD, PhD,[a] Rajendra G. Mehta, PhD,[c] Rajeshwari R. Mehta, PhD,[c] William H. Hall, MD,[a] Philip S. Boerner, MA,[a] Laurel A. Beckett, PhD,[b] Srinivasan Vijayakumar, MD, FACR,[a,c]

ABSTRACT

Prostate cancer continues to be a significant source of morbidity and mortality among older men. One possible means of reducing its impact on overall health and vitality is via cancer chemoprevention, both in the population that is unaffected but at some risk and in those who have undergone some form of curative therapy after the onset of the disease. Chemoprevention holds significant promise, but large phase III clinical trials evaluating chemopreventive agents in prostate cancer can require vast numbers of enrollees and require the commitment of significant financial resources and time before any therapeutic benefit may become apparent. One technique to shorten the time required for chemoprevention clinical trials is to use surrogate endpoint biomarkers in place of the currently used actual endpoints of cancer incidence or survival. The validation of such surrogate endpoint biomarkers will require small, well-designed phase I and/or II trials to accumulate data on the modulation of the surrogate biomarkers and the endpoints of cancer incidence or survival by the chemopreventive agent. Careful statistical correlation and clinical validation of the data will then allow us to justify the use such surrogates in place of the actual endpoint in large, randomized trials, potentially shortening trial duration, improving financial efficiency, and accelerating approval of the chemopreventive agent. To that end, we first review the theoretical construct of cancer chemoprevention trials with particular reference to prostate cancer. We thereafter describe the design of a small, randomized, double-blinded, placebo-controlled phase I/II clinical trial of an analogue of vitamin D, vitamin D_5, which we believe could serve as a model for data accumulation on surrogate biomarkers and correlation with other clinical endpoints. *(Cancer J 2004;10:357–367)*

KEY WORDS

Chemoprevention, vitamin D, prostate cancer, radiation therapy, randomized clinical trial, phase I/II

From the [a]Department of Radiation Oncology and the [b]Division of Biostatistics, Department of Public Health Sciences, School of Medicine, University of California at Davis, One Shields Avenue, Davis, California, and the [c]Department of Surgical Oncology, School of Medicine, University of Illinois at Chicago, Chicago, Illinois.

Received on September 22, 2004; accepted for publication October 10, 2004.

No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this article.

Reprint requests: Srinivasan Vijayakumar, MD, FACR, Professor & Chairman, Department of Radiation Oncology, U C Davis Cancer Center, 4501 "X" Street, Suite G126 Sacramento CA 95817.

E-mail: srinivasan.vijayakumar@ucdmc.ucdavis.edu.

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Prostate cancer, the risk factors for which include older age, family history, ethnicity, and race,[1] is one of the more common cancers afflicting men in the United States and Western Europe. One autopsy study, for instance, documented prostatic carcinomas in as many as 29% of men between the ages of 30 and 39 years and in 63% of those between the ages of 60 and 69 years.[2] Because of the often decades-long latency period for progression from normal tissue to prostate cancer, it is believed that effective chemoprevention could be a viable means of reducing the incidence of prostate cancer. To that end, large, randomized, double-blinded, phase III chemoprevention clinical trials, such as the Prostate Cancer Prevention Trial (PCPT)[3-5] and the Selenium and Vitamin E Cancer Prevention Trial (SELECT),[6,7] were initiated. However, the major endpoint in these large studies is the onset of prostate cancer, which, despite its significant public health impact, has only a low annual incidence (0.27% in men ≥ 34.4 years of age).[8] This, coupled with prostate cancer's long latency period, may necessitate prolonged trial monitoring before any reduction of prostate cancer incidence is demonstrated. Improvements in trial design and efficiency are thus eagerly

awaited, not merely in prostate cancer studies but in other types of cancer studies as well.

Short and efficient phase III trials could be theoretically accomplished with fewer resources and patients if validated surrogate endpoint biomarkers (SEBs) that were accepted by the US Food and Drug Administration (FDA) were available in addition to the currently accepted endpoint of actual disease presence. It is also critical that SEBs be initially examined clinically in small phase I and II trials before they are used and further validated in phase III trials.[9] Thus, in the case of prostate cancer, investigators would test the efficacy of current or new chemopreventive drugs and at the same time gather valuable data on potential SEBs, such as prostate specific antigen (PSA) modulation, prostatic intraepithelial neoplasia (PIN) progression, and molecular/genetic biomarkers in small, randomized, phase I/II studies. Hence, the SEBs' statistical and clinical correlation with, and their predictive potential for, the endpoint of prostate cancer per se can be more strongly established. Moreover, important design elements, such as population selection, randomization, quality and type of SEBs, and quality of life (QOL) parameters can be carefully integrated and optimized for the type of cancer and the drug being investigated.[8] Such multipronged initial efforts could potentially lead to shorter and more efficient phase III trials.[10]

The active form of vitamin D, 1α,25-dihydroxycholecalciferol [$1\alpha,25(OH)_2D_3$ or vitamin D_3], or calcitriol, because of its antiproliferative and differentiation-inducing properties, has been extensively investigated as a potential chemopreventive agent. Some of these studies have detected promise in cutaneous, colorectal, breast, and prostate cancer chemoprevention.[11-14] Because of the significant toxicity of vitamin D_3—secondary to hypercalcemia induction at pharmacologic doses—analogues less likely to induce hypercalcemia have been designed and developed for use in cancer chemoprevention. These analogues have anti-proliferative potential at least equal to if not greater than that shown by vitamin D_3.[15] One such analogue that has undergone significant preliminary testing is 1α-hydroxy-24-ethyl-cholecalciferol [$1\alpha(OH)D_5$ or vitamin D_5]. Reportedly, vitamin D_5 is the least toxic of the vitamin D series of compounds D_2 through D_6 and has been examined in several preclinical studies.[16]

In this paper, we briefly review the concepts underlying chemoprevention, clinical trials, and surrogate endpoints before detailing our experience in designing a combined phase I/II randomized clinical trial to evaluate the effectiveness of 1α-hydroxy-24-ethyl-cholecalciferol [$1\alpha(OH)D_5$] in prostate cancer chemoprevention. Our short overview of each of these is limited primarily to the role played by each in prostate cancer.

CHEMOPREVENTION

Since its introduction in 1976,[17] chemoprevention has been accepted as an essential ally in cancer therapy.[18,19] Generally, cancer chemoprevention agents function via three broad mechanisms: their carcinogen-blocking, antioxidant, and antiproliferative/antiprogression activities.[20] Depending on the type of cancer being targeted, chemopreventive agents can be dietary factors, nutritional supplements, hormones, intra- or extracellular receptor modulators, growth factor inhibitors, anti-inflammatory agents, and specifically directed gene therapy, among others. In its broadest sense then, cancer chemoprevention attempts to use natural, synthetic, biologic, or chemical agents to suppress, reverse, or prevent carcinogenic progression to invasive cancer.[21]

Although simple in concept, chemoprevention harbors significant promise in cancer control because it inhibits the formation of the precancerous state and impedes or halts carcinogenic progression. Chemoprevention clinical trials have been initiated or have been considered for virtually all cancers, including bladder cancer,[22,23] prostate cancer,[24,25] gastric cancer,[26,27] hepatocellular carcinoma,[28,29] breast cancer,[30,31] head and neck cancer,[32,33] colorectal cancer,[34-36] and lung cancer.[37,38] Thus, one could conceivably argue that chemoprevention will become an increasingly important tool in our therapeutic armamentarium against all types of cancer, especially those with long latency periods from mutagenesis to cancer.

Although chemoprevention itself may be a novel, yet simple, concept, the epidemiologic, experimental, and/or other preclinical studies considered necessary to provide evidence that a particular drug or intervention can be beneficial in the prevention of a particular type of cancer are complex. Moreover, once a beneficial effect has been established by, or at least inferred from, these studies, one then faces the difficult task of designing appropriate clinical trials to test the interventions.

In the next section, we very briefly review some of the concepts underlying clinical trials before delving into the relationship between actual endpoint biomarkers and SEBs.

CLINICAL TRIALS

Clinical trials represent today's frontiers of medicine. Each properly conducted and completed trial, regardless of outcome, advances our understanding of disease processes and patient treatment options in a setting that is clinically as safe and as devoid of bias as possible. Clinical trials are routinely classified as being phase I, II, or III. A phase I trial is very often unrandomized, enrolls a small number of patients, and focuses primarily on

patient safety, drug doses, pharmacokinetics, and pharmacodynamics, as well as a very limited estimation of patient response to the treatment. The phase II trials, which are sometimes randomized, estimate treatment efficacy at a more limited range of doses, while continuing data collection on adverse events. Thus, a phase II trial, with only a modestly larger number of patients, focuses more closely on the clinical benefit to be derived from the drug or the intervention. Such studies also provide essential guidance regarding the degree of clinical and statistical response, if any, that one could anticipate from the drug in a phase III trial.[9] Moreover, if an intervention has been documented in preclinical studies to have only nominal side effects, phases I and II could potentially be combined into a single trial. In the phase III trials, however, large numbers of subjects are sought and then randomly assigned to various treatment and control arms to estimate the benefits of the intervention with the expectation that findings may be generalized and applied to the population from which the trial participants were derived.[39]

In cancer, clinical trials are routinely organized to evaluate a therapeutic or a chemoprevention strategy. Ideally, chemoprevention trials would target individuals who are currently healthy or who are healthy but have a significantly higher than normal risk of developing cancer in the future. The underlying benefit to such a patient population lies in the anticipated reduction in the incidence of the cancers being targeted. Examples of such targeted patient populations include

- Patients with head and neck precancerous states, such as leukoplakia for head and neck squamous cell cancers[33]
- Smokers for lung cancer[40]
- Heavily sun-exposed individuals for various skin cancers[41]
- Individuals with colonic adenomas for colonic cancer[42,43]
- Prostate biopsy-negative men with varying degrees of PIN but only modestly elevated serum PSA levels[4,5]

In addition, some chemoprevention trials are opened to cancer patients who have undergone or will soon undergo some form of therapy that is considered curative, such as surgery or radiation therapy (RT). In such patients, the trials test the hypothesis that the planned chemoprevention will supplement or even augment the curative therapy by reducing or eliminating the likelihood of recurrence or of a second primary tumor. Such tertiary patient populations have included those with head and neck squamous cell cancers,[44] breast cancer,[45] lung cancer,[46] colorectal cancer,[47] or prostate cancer.[48]

Hence, depending on patient selection, chemoprevention therapies can be targeted toward primary, secondary, or tertiary prevention. In primary prevention, it can occasionally be difficult to recruit healthy individuals into chemoprevention trials and maintain their compliance with the treatment regimen because their self-perception of personal risk may be low. In addition, any intervention or chemopreventive drug in this population must have minimal side effects. Sometimes, too, phase I chemoprevention studies are performed in the tertiary prevention population because the safety of the test drug may not yet have been adequately established for use in the healthy or healthy but still higher-risk population. In patients already diagnosed with cancer, a greater degree of uncertainty about the drug's toxicity may be considered acceptable, given its potential benefit.

However, therapeutic cancer trials are exclusively directed toward patients who have a diagnosis of cancer and are awaiting therapy. Such trials often compare the efficacy of different treatments or examine the superiority of one type of treatment over another. On occasion, they may investigate the use of an experimental drug or therapy on a seemingly incurable form or stage of cancer. The endpoints in these studies can include QOL improvements, length of disease-free survival, extent of local or systemic control of disease, or outright cure. Patients in therapeutic trials are often seriously or even terminally ill. Yet, because these early investigational or untried interventions may represent the only clinical option available to palliate symptoms, prolong life, induce disease remission, or cure the disease, a higher degree of drug toxicity would be considered an acceptable risk, given the potential benefit. Similar criteria also underlie the bases of patient selection in the therapeutic trials of other medical specialties.[49]

The key element of a clinical trial then, apart from its targeted patient population and the interventions planned, is the disease endpoint it is designed to monitor. In the subsequent section, we review briefly the basic principles of endpoints and SEBs before proceeding to discussion of the vitamin D_5 clinical trial.

ENDPOINTS AND SURROGATE ENDPOINT BIOMARKERS

The definitive endpoints of any disease are final clinical outcomes that are relevant to the patient and/or the health community. These may include death, loss of function of an organ, a diagnosis of cancer, and a cardiac event. SEBs are alternatives to the actual endpoint, the modulations in which correlate with and predict, statistically and clinically, the true endpoint. Such SEBs are as a rule attained faster, require less invasive monitoring, and are less costly to observe than the true end-

point.[50-52] Some selected SEBs for neoplastic and nonneoplastic diseases include

- Bronchial metaplasia for lung cancer[53]
- Plasma cholesterol levels for the endpoint of a cardiac event[54]
- Cervical intraepithelial neoplasia for cervical cancer[55]
- CD-4 cell counts and plasma viral loads for death or opportunistic infections in human immunodeficiency virus–infected patients[56]
- Changes in colonic adenoma number/histology for the likelihood of colonic cancer[8]
- Intraocular pressure for vision loss in glaucoma[57]

In chemoprevention trials, SEBs are particularly useful for estimating the effects of preventive interventions on the endpoint of cancer incidence. Especially in phase II chemoprevention trials, appropriate SEBs may permit rapid preliminary assessment of efficacy, dose response, and suitability for progression to phase III trials. In the case of prostate cancer, potential SEBs include serum PSA level, PSA doubling time, serum alkaline phosphatase level, histochemical/molecular monitoring of apoptotic biomarkers, changes in degree or new occurrence of PIN, cell/nuclear morphometry, chromosomal changes, and QOL parameters. Any modulations noted in SEB measures must actually predict increased/decreased prostate cancer risk, and these must be appropriately validated before the chemopreventive efficacy is accepted and used. This validation necessitates the fulfillment of four criteria by the SEB:[9]

1. The SEB is differentially expressed between normal and tumor tissue.
2. The SEB can be modulated by the planned intervention.
3. The SEB modulation by the intervention can be correlated with clinical response.
4. The SEB modulation by the intervention correlates with long-term cancer development.

Validation of SEBs for use in clinical trials is statistically a complex and demanding task whose methodology has been detailed elsewhere.[58,59] However, brief mention is made here of SEB validation with respect to prostate cancer chemoprevention.

The optimal SEB for any cancer will lie in the pathway leading to the endpoint and will directly affect the incidence of the endpoint. The ideal means of establishing the validity of a SEB as a substitute for the actual endpoint is by conducting a clinical trial with the endpoint that the SEB is designed to replace.[51] However, validating such a benchmark is impractical because cancer can take decades to develop. Hence, initial statistical correlation will more than likely be extracted from other in vitro, in vivo, or epidemiologic studies in which the SEB was also monitored in addition to the actual endpoint. Before an SEB is selected for further study, an estimate of cancers that can be attributed to the SEB must be made. This "attributable proportion" or AP is represented by the formula:

$$AP = S\,(1 - 1/R)$$

where R = relative risk and S = sensitivity. A value close to 1.0 suggests that the SEB under evaluation is very likely to lie in the pathway leading to the cancer endpoint. In contrast, values ≤ 0.5 for the AP would suggest that 50% or less of the cancers can be attributed to the SEB.[9]

In addition, under the null hypothesis, the SEB must yield the same result as the true endpoint. This fundamental criterion and others regarding the statistical principles for the use of SEBs were initially articulated by Prentice.[60] Those statistical beginnings have been gradually refined as the sophistication of statistical methodology improved.[52,61,62] One refinement, for example, is the concept that any changes in SEB must actually meet the requirement of "predicting" the likelihood of the actual endpoint rather than merely "correlating with" it.[61] Thus, before any conclusions of therapeutic efficacy can be drawn from SEB modulation by an intervention, such modulations of the SEB must also concordantly predict the effect on the actual endpoint. To rephrase this as an example familiar to prostate cancer, any intervention that reverses or decreases the rate of transformation to high-grade PIN should also actually translate into a decrease in prostate cancer incidence.

Even when SEB modulation by an intervention appropriately predicts the endpoint in preliminary studies, its validity can be called into question after large, randomized trials produce contradictory outcomes, such as hormone replacement therapy and cardiac disease in postmenopausal women.[63] Hence, even meticulous prephase III trial substantiation of an SEB cannot guarantee that it will perform in a similar manner in the randomized drug/placebo treatment protocol of an actual phase III trial.[61] Thus, SEBs to be used in phase III trials must be carefully selected. Even if their use is meticulously validated before they are selected, any reliance on them must be made with the stipulation that they can be quickly superseded by newly accumulating evidence.

Having briefly reviewed the concepts of chemoprevention, clinical trials, and SEBs, we now describe our experience in the design of a randomized phase I/II clinical trial to test the efficacy of a vitamin D analogue in patients with prostate cancer.

Appendix 7

DESIGNING A POSTRADIATION THERAPY CHEMOPREVENTION TRIAL USING VITAMIN D_5

Rationale

RT and radical prostatectomy (RP) are the two major treatments for nonmetastatic prostate cancer, with essentially no difference in long-term patient outcomes.[64] At diagnosis, approximately 30%–50% of patients with nonmetastatic prostate cancer elect to undergo RT instead of RP. Of these, ~ 30%–40% at some point face biochemical and/or clinical failure despite this treatment option. Such failure is associated with poor prognostic factors on initial presentation. These prognostic factors, which include patient ethnicity, American Joint Committee on Cancer disease stage, pretreatment PSA level, pre-RT PSA nadir, and Gleason score, are each independent predictors of PSA relapse-free survival.[65-67]

Patients who do not respond to RT very likely do so because of clonal growth of radioresistant cancer cells or because of malignant clones arising from precancerous cells. In RT, because the prostate gland is permitted to remain in situ, the intrinsic "stimuli" that initiated mutagenesis and allowed progression to the original cancer can continue to exert their influence on the prostatic cells. Thus, the potential for recurrence is present for the remainder of the patient's life. After diagnosis of recurrence or of biochemical failure, these patients may face the grim prospect of undergoing continuous or intermittent androgen blockade, with all its associated side effects, including hot flashes, loss of libido, erectile dysfunction, tiredness, gynecomastia, and loss of bone mineral density, essentially for the rest of their lives. Very rarely, such a patient may choose to undergo salvage RP instead, if that option is offered. However, its benefits are uncertain, its complication rate significant, and its long-term outcome unknown. Hence, it would be extremely beneficial to the patients with prostate cancer who have undergone RT (perhaps even those who have undergone RP) if a chemopreventive agent that could delay or prevent the onset of biochemical failure or cancer recurrence were available.

Vitamin D_3, or calcitriol, has antiproliferative and differentiation-inducing properties that make it a potential chemopreventive agent for multiple cancers, including prostate cancer. Because of its hypercalcemic toxicity at pharmacologic doses, however, its less toxic analogues are now appearing to be better suited for a role in chemoprevention. One such analogue is vitamin D_5, or 1α-hydroxy-24-ethyl-cholecalciferol [1α(OH)D_5]. This compound, designed by Mehta and colleagues,[68] and manufactured under FDA "good manufacturing practice" guidelines, has been slated for use in an upcoming breast cancer phase I trial. Preclinical toxicity studies have also been completed in at least two separate species as required by the FDA.

Design Considerations

The major design considerations for chemoprevention trials in humans include identifying a chemopreventive agent, defining the type of clinical study (phase I, II, or III) and its duration, selecting a target population, selecting biomarkers for toxicity monitoring, choosing appropriate SEBs for disease monitoring, and using statistical modeling.[8,39,69] Within each category, however, design criteria must incorporate patient safety guarantees, appropriate statistical principles, and sufficient flexibility to modify drug/intervention regimens and to respond to institutional review board concerns.

Before initiating the design phase for our study, we carefully reviewed the design details underlying two recent large-scale, randomized phase III trials (PCPT and SELECT). After this review, we incorporated the features that, in our estimation, would optimize our design and maximize the potential for a clinically and statistically valid outcome of this randomized phase I/II study.[70]

The Prostate Cancer Prevention Trial (PCPT) is a large, randomized, double-blinded, placebo-controlled, period prevalence, and point prevalence study aimed to determine the usefulness of finasteride in reducing the incidence of prostate cancer. Begun in 1994, its design incorporated a three-month enrollment period during which all participants received the placebo before they were randomly assigned into treatment and control arms. In addition to any diagnostic biopsies performed during the 7-year treatment phase, all participants surviving to the end-of-study were expected to undergo a prostate biopsy. Its "period prevalence" design for the endpoint of prostate cancer incidence was decided on after much discussion among the study investigators. This allowed calculation of overall prostate cancer incidence during the 7 years of the trial, as well as "point prevalence" of prostate cancer at the 7th-year biopsy. Study participants were males > 55 years of age with normal PSA levels (≤ 3 ng/mL) and no other significant comorbid disease.[3]

This study was halted ~ 15 months before scheduled completion when its monitoring committee determined that the robust statistical differences between the treatment and the placebo groups were unlikely to improve in the time remaining. In the recently published summary of the trial findings, it was observed that finasteride decreased the period prevalence of prostate cancer by 24.8% over the 7-year period. However, the unexpected finding that a significantly higher percentage of prostate tumors discovered in the finasteride-treated group were

of Gleason grade ≥ 7 provided a sobering and thought-provoking counterpoint to the reduction in overall prostate cancer incidence.[4,71]

The SELECT study, designed to test the effect of selenium and vitamin E on prostate cancer incidence, differs from the PCPT primarily in not having an enrollment period in which all participants received a placebo and in not requiring an end-of-study prostate biopsy. In addition, it uses community standards of medical care in diagnosing the endpoint of prostate cancer; that is, within-study biopsies are not mandated and are performed only at the discretion of the treating physician.[7] Moreover, it differentiates by race in its enrollment criteria, reflecting established racial differences in prostate cancer incidence, by permitting African-American men to begin enrolling at ≥ 50 years, whereas others could begin at ≥ 55 years of age. Enrollment in this randomized, placebo-controlled, double-blinded study began in 2001, and its findings are anticipated after the study ends in 2013.[7]

Having reviewed these two major studies, we proceeded then to design a randomized, double-blinded, placebo-controlled chemoprevention trial targeted toward patients with nonmetastatic prostate cancer who had undergone RT.

STUDY DESIGN CONSIDERATIONS

Chemopreventive Agent

There were no special considerations involved in our selection of vitamin D_5; it has been under active and collaborative investigation between investigators at the University of California at Davis and the University of Illinois at Chicago.[16] Vitamin D_3, the parent analogue, has been used in several small clinical trials, although some of them have had to be limited because of hypercalcemia, the major obstacle to the use of vitamin D_3 in pharmacologic doses. These studies on vitamin D_3 or its analogues, which began in 1995,[72] have tried different dosing paradigms or have used vitamin D_3 in combination with other drugs in an effort to reduce its dose[73,74] and minimize hypercalcemia. Our main criteria for selecting the analogue vitamin D_5 were its antiproliferative and differentiation-inducing activities, coupled with its nontoxicity. Because these criteria had been well documented in preclinical cell culture studies and because any toxicity in rats and beagles was not apparent until at ~ 10 times the planned clinical trial dose of 10 μg/day,[16,70] we considered its use in this context to be safe. Moreover, we incorporated dose de-escalation criteria into the trial design to overcome concerns regarding adequate protection of any patient developing symptoms of toxicity.[70]

Target Population

Traditionally, the ideal population in a chemoprevention phase I or II trial for an hitherto untested, but minimally toxic, drug would be those seeking tertiary protection after some type of "curative" therapy. It may also be of benefit if it is given before the curative treatment. However, it would not be used in patients with metastatic cancer because the window of opportunity for chemoprevention is no longer present (unless a curative therapy were available and was planned to be used). Similarly, because of uncertainty in pharmacokinetics, pharmacodynamics, and toxicity in humans, its use may be inappropriate in patients in the primary prevention category (healthy general population) and is perhaps only marginally acceptable for use in those in the secondary prevention category (healthy but at high risk). Because patients in both of these categories have yet to be diagnosed with cancer, administration of drugs known to be toxic, of unknown toxicity, or even of mild toxicity can be open to ethical challenge, given that the potential benefit or benefits to these patients is unclear. In attempting to appropriately address these considerations, we decided to enroll only patients who fell into one specific category: those who had had their prostate cancer treated "curatively" by RT and thereafter needed chemoprevention to prevent or delay the onset of new cancers, recurrences, or biochemical failure.[70]

Patients with prostate cancer have generally been stratified into low-, intermediate-, or high-risk cohorts for biochemical failure or cancer recurrence on the basis of prognostic factors, such as disease stage, PSA level, and Gleason score at initial disease presentation.[75] In one study using these criteria in patients who had undergone RT, the 5-year PSA relapse-free survival was ~ 60% in the intermediate-risk group and ~ 40% in the high-risk group.[65] As the impact of race on prostate cancer incidence became readily apparent, more recent stratifications have included ethnicity in addition to the Gleason score, PSA level, and pathological stage at the time of presentation, to stratify patient risk into very-low-, low-, high-, or very-high-risk categories.[67] In patients who have undergone RP, these authors then calculated 85%, 66%, 51%, and 21%, 7-year disease-free survival in the very-low-, low-, high-, and very-high-risk groups, respectively. Thus, inasmuch as ~ 30%–50% or more of the patients who elect to undergo RT or RP, especially those in the intermediate- and higher-risk groups, may demonstrate either biochemical or clinical failure of their "curative" therapy within 5–7 years, any effective chemopreventive agent that decreases these percentages would be a valued addition to treatment options.

This targeted population of patients with prostate cancer may also obtain an added benefit from their trial

participation with the use of hormonal therapy after RT, that is, at the time of PSA relapse. Such therapy has been shown to improve 5-year disease-specific and biochemical disease-free survival.[76,77] However, the major concern with beginning early hormonal therapy is the increased risk of earlier development of a hormone-refractory state, especially after the development of metastases. Thus, although hormonal therapy may improve metastasis-free survival, patients may actually be hormone refractory when metastases do develop.[78] Moreover, there is often only a short period between PSA recurrence, bone metastases,[79] and detection of occult nodal disease by scintigraphy.[80] Thus, it could be argued that because of the more intensive monitoring of PSA parameters, the fitting of these parameters into the ASTRO[81] and/or Jani et al[82] criteria, and the pre- and poststudy biopsies, we, and the patients in the trial, will be better positioned to

- Detect any occult prostate cancer in the trial participants
- Determine more precisely the optimal time for the initiation of hormonal therapy

Because the ideal PSA thresholds for initiating delayed hormonal therapy have yet to be established,[78] this study may provide exciting new information that will permit the pinpointing of an appropriate time to begin hormone therapy and potentially provide the parameters for the design of clinical trials involving hormone therapy. Moreover, trial participants may benefit by being able to start hormone ablation therapy at a more appropriate point in the disease timeline, possibly contributing to their longer survival and perhaps decreasing the likelihood of developing a hormone-resistant state.

Study Design and Duration

The simplest clinical study design is the randomized one-way layout, in which one study arm is compared individually against another study arm.[8] In our case, having only two randomized arms—vitamin D_5 and placebo—the one-way layout was therefore an appropriate design choice. In addition, because vitamin D_5 has not demonstrated any toxicity except beginning marginally at ~ 10 times the experimental daily dosage used here, any potential toxicity at the experimental dosage could most likely be considered nominal. We elected therefore to combine both phase I and phase II into one randomized phase I/II trial to assess the toxicity, pharmacokinetics, pharmacodynamics, and treatment efficacy of vitamin D_5 in prostate cancer chemoprevention.

A major strength of this study lies in its use of randomization. Randomization eliminates selection biases and allows application of various parametric and nonparametric statistical tests to be applied to the results that will be obtained. In addition, unknown prognostic factors can be better controlled.[8]

To assess and ensure patient compliance, we have incorporated a prerandomization "run-in" period as was also used in the PCPT trial.[3] During this 1-month period, all enrolled patients will take the placebo and keep a pill calendar/diary, which, together with the medication containers, will be carefully monitored during the once-weekly clinic visits. Any degree of compliance totaling < 90% over the month will be grounds for excluding the patient from the study.

After randomization, patients will be monitored medically once a week for 1 month, transitioned to monthly monitoring with weekly telephone calls if this is clinically appropriate and thereafter moved to once every 4 months (Fig. 1).

Biomarkers for Toxicity

The primary toxicity of vitamin D_3 lies in its ability to induce hypercalcemia. This is a major concern in the use of both vitamin D_3 and its analogues.[16] Although vitamin D_5 has not thus far demonstrated hypercalcemia at the doses planned for use in this study, assessing drug toxicity represents a major portion of any phase I study. To that end, serum chemistries, serum albumin, parathyroid hormone (PTH), urine chemistries, and patient questionnaires regarding symptomatology will be closely followed weekly, monthly, and then every 4 months.

Because vitamin D_3 is fat soluble, and the same could be expected of its analogue vitamin D_5, any toxicity may not be apparent until its stores in the body fat have accumulated sufficiently. This formed the basis of our rationale for the 2-year treatment and follow-up phase. This also was a reason for selecting a somewhat healthier cancer patient population for this study because it is

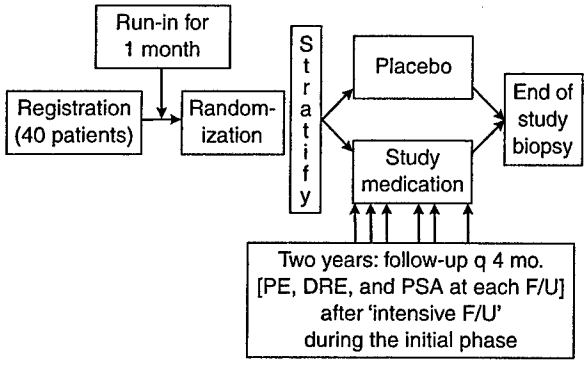

FIGURE 1 Flow sheet depicting timeline, monitoring intervals, and monitored parameters for the vitamin D_5 clinical trial.

highly unlikely that participants with metastatic cancer will survive to provide at least 2 years of data accumulation necessary to demonstrate drug safety for use in primary chemoprevention trials.

Step-wise dose de-escalation protocols for reducing the daily dosage from 10 to 5 to 2.5 μg/day have been incorporated for use in patients demonstrating toxicity via serum chemistries or symptom diaries. Appropriate parameters for withdrawal of a patient from the study because of undue toxicity or other clinically valid reasons have also been included.

Endpoint and Surrogate Endpoint Biomarkers

Several endpoints will be monitored in this study, including vitamin D_5 toxicity, changes in vitamin D receptor number and distribution, biochemical failure indicated by three consecutive increases in PSA,[81,83] biochemical failure as defined by Jani et al,[82] incidence of cancer in the beginning- and end-of-study biopsies, presence of metastatic cancer, QOL assessment, and surrogate biomarker profile.

Potential SEBs that will be assessed in this study include PSA and its associated parameters, such as PSA velocity and doubling time. We also plan to use the prostate biopsy samples as a source of tissue and molecular markers that may potentially function as SEBs. These will include the various grades of PIN; Gleason score; chromosomal markers, especially in the 8q region; molecular markers of apoptosis, such as *Bcl-2, Bax, Bcl-x, PTEN*, and *AKT*; newer molecular markers with prognostic potential, such as *Ki-67*, thymosin-β15, fatty acid synthase, and E-cadherin; and QOL parameters. Potentially, the DNA, RNA, and complementary DNA derived from the tissue samples will be amenable to high-through-put screening techniques using array systems, as has already been demonstrated by other investigators.[84-87] Thus, in correlating the SEBs with the actual endpoint of prostate cancer recurrence or biochemical failure, we anticipate the day when a panel of SEBs or a single SEB may be deemed comparable to an actual endpoint for clinical purposes by the FDA.

Statistical Modeling

The statistical analyses will derive from accepted methodologies under the guidance and expertise of a faculty statistician. Comparisons will be made between each arm using Fisher's exact test for quantitative data with "intent-to-treat" analyses. Nonparametric data will be assessed using Wilcoxon rank sum or log-rank sum, as appropriate. For the QOL assessments, performed via questionnaires every 4 months, we will fit previously described regression models for longitudinal data.[88]

The number of patients we plan to enroll allows sufficient statistical power to detect a decrease in prostate cancer recurrence from ~ 50% in the placebo group to ~ 10% in the vitamin D_5 treatment group. Correlation between longitudinal measures of potential SEBs and prostate cancer recurrence will be assessed in several ways. We will examine differences in time to recurrence using survival models with a time-varying covariate. In addition, we will use repeated measures regression models[88] to determine whether the approach based solely on using change in SEB over time can predict recurrence of disease and can distinguish between the recurrence rates in the two groups. We will test the ability to distinguish recurrence rates by including an indicator term in the model for recurrence; other, more complex, statistical models will examine time to recurrence or whether different recurrence rates in the two groups could be detected. As we have noted previously, the statistical modeling and validation of SEBs are a mathematically complex and demanding endeavors. Readers are referred to the references cited previously[58,59] for more details regarding these statistical analyses.

Summary

To summarize, in our randomized, placebo-controlled phase I/II chemoprevention clinical trial design, we anticipate recruiting 40 patients (20 for each arm) who are all within ~ 12–60 months of completion of RT for prostate cancer. They will be randomly assigned to either the D_5 or the placebo arm after 1 month of placebo administration (the run-in period) to assess the quality of patient compliance. All patients will undergo a pretreatment biopsy, receive baseline clinical staging, and undergo serum PSA level measurement. Serum chemistries, serum albumin, serum PTH, and urine electrolytes will also be measured. For the first month at least, all subjects will be monitored weekly via serum chemistries and albumin levels. Thereafter, individuals who continue to demonstrate stable and nonhypercalcemic serum calcium levels will be monitored with weekly phone calls and continue with monthly clinical and laboratory assessments of serum chemistries, albumin levels, PTH levels, and urine electrolyte levels. Individuals who continue to demonstrate stable serum calcium levels at 4 months will then transition to a 4-month monitoring cycle with biannual measurements of serum PTH level (see flow sheet in Fig. 1).

We anticipate being able to monitor all the study participants for a minimum of 2 years for the trial except in the event of patient death or medically justifiable inability to continue in the protocol or patient's voluntary withdrawal from the trial. However, routine and extended follow-up care will continue as long as the enrollees remain patients of the University of California at Davis Cancer Center.

The strengths of this trial lie in its randomization and

placebo control, the optimization of target population selection, the assessment of SEBs, and its use of end-of-study biopsies to confirm and provide correlative evidence of outcomes. We hope that this combined phase I/II trial will serve as an useful model for small, efficient clinical trials that assess chemopreventive potential as well as accumulate valuable data on the use of SEBs.

CONCLUSION

In this paper, we have briefly considered some of the core concepts underlying chemoprevention, clinical trials, endpoints, and SEBs. Of the myriad forms and types of cancer facing our patients, we elected to direct our attention primarily to the goal of prostate cancer chemoprevention. To that end, we have discussed herein our experience in designing an institutional review board–approved, small, selective, combined phase I/II randomized, placebo-controlled, double-blinded clinical trial, that uses vitamin D_5 and may serve as a model for data accumulation about selected SEBs and cancer recurrence. Importantly, the study includes end-of-study biopsies that all participants undergo to ensure that tissue samples are available for correlation with the SEBs used in the study, as well as for the analysis of newer genetic/molecular markers that could potentially serve as SEBs. To accomplish these aims, we elected to obtain study participants from a high-risk population of patients with prostate cancer who, because of poorer prognostic factors on initial presentation, may face a higher incidence of biochemical failure or cancer recurrence after RT. We anticipate that the toxicity and clinical data gathered herein, as well as in the other studies using vitamin D and its analogues, will accelerate the day when vitamin D_5 will become available as an effective and safe chemopreventive agent for all men.

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Appendix 7

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CLINICAL PROTOCOL

A Phase I/II Double-Blinded, Randomized Clinical Trial to Prevent/Delay Biochemical and Clinical Failure in High-Risk, Non-Metastatic Prostate Cancer Patients After Radiotherapy, Using 1α-Hydroxyvitamin D5 Versus Placebo: A Tolerance-Finding and Intermediate Biomarker Response-Seeking Study

University of California, Davis Medical Center (UCDMC)
and
University of Illinois at Chicago (UIC)

A Department of Defense-Funded Study

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Principal Investigator:

Srinivasan Vijayakumar, M.D.	Radiation Oncology, UCDMC

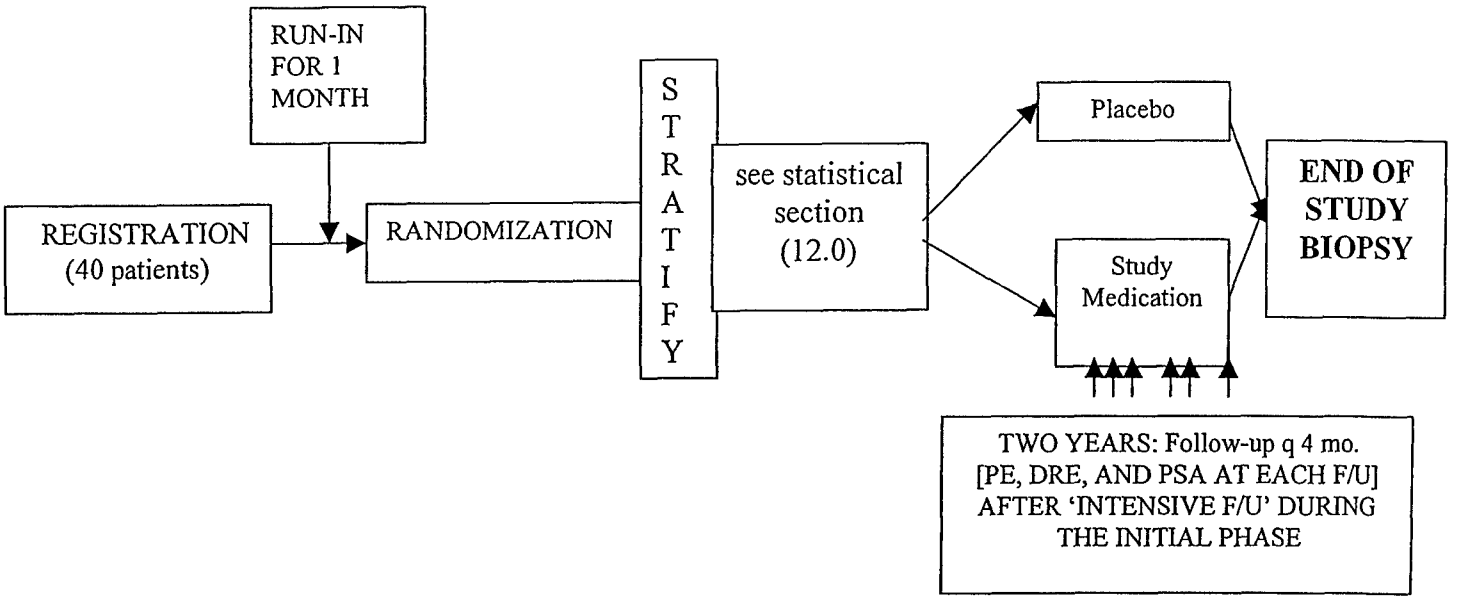
Co-Investigators:

Ralph W. deVere White, M.D	Urology, UCDMC
Samir Narayan, M.D.	Radiation Oncology, UCDMC
Janice K. Ryu, M.D.	Radiation Oncology, UCDMC
Paul Gumerlock, M.D.	Hematology & Oncology, UCDMC
Laurel Beckett, Ph.D.	Epidemiology & Preventive Medicine, UCDMC
Ralph Green, M.D.	Pathology, UCDMC
William C. Baker, Jr., M.D.	Urology, VA Mather
Rajendra G. Mehta, Ph.D.	Surgical Oncology, UIC
Rajeshwari R. Mehta, Ph.D.	Surgical Oncology, UIC
Alan Diamond, Ph.D.	Human Nutrition, UIC

Clinical Research Associates

Cathy Hollister	Radiation Oncology, UCDMC
Cheri Grelle	Radiation Oncology, UCDMC
Clinical Research Nurse (To Be Hired)	Radiation Oncology, UCDMC

STUDY SCHEME



1. PURPOSE, METHODS, AND PROCEDURES

PURPOSE

The prostate gland is left *in situ* after radiation therapy; hence, the phenomenon of "field changes" and the factors responsible for prostate cancer initiation, promotion, and progression continue to operate in the prostatic cells. This results in radiation therapy failure [PSA and/or clinical] in 30-50% of patients. We hypothesize that treating patients with a relatively non-toxic chemopreventive and therapeutic agent, 1α-hydroxyvitamin D5, post-radiation therapy will prevent or delay the local recurrence of prostate cancer in radiation-treated prostate cancer patients. This will enhance their outcome results, including the quality of life (QOL) in patients receiving radiation therapy (RT) as the primary modality. In this study we are targeting high and intermediate risk patients, who are more likely to be in the 30-50% of patients that will have radiation therapy failure (see inclusion and exclusion criteria).

BACKGROUND

NON-METASTATIC PROSTATE CANCER, ADVERSE PROGNOSTIC FACTORS, AND THE USE OF RADIOTHERAPY

Non-metastatic Prostate Cancer and the Prognostic Factors that Affect the Outcomes within that Stage

The American Cancer Society estimates that 220,900 new prostate cancers will be diagnosed in the year 2003 in the U.S. and 28,900 men will die of prostate cancer in 2003 [Jemal et al., 2003]. Prostate cancer constitutes 33 % of all cancers among men and 10 % of cancer-related deaths [Jemal et al., 2003]. Over 90 % of cases diagnosed between the years 1992 and 1997 were non-metastatic, representing a stage migration, influenced by prostate specific antigen [PSA]-based screening efforts [Vijayakumar et al., 1998; Jani et al., 2001]. Yet, many patients with localized prostate cancer carry adverse prognostic characteristics, and these patients carry higher chances of failure and development of metastases [Chuba et al., 2001] and death [Satariano et al., 1998]. For instance, in a National Patterns of Care [POC] study, a higher T-Stage, Gleason Sum [GS], and pre-treatment PSA levels predicted worse outcomes. In the Univariate analysis, cause-specific failure was significantly lower for higher T stage ($p = 0.014$), GS ($p = 0.001$), and pretreatment PSA ($p = 0.0004$); overall survival was significantly lower in patients with higher T stage ($p = 0.047$) or GS ($p = 0.0191$). This study had 600 patients treated in 71 institutions in the U.S. Other individual institutional data also suggest the prognostic importance of those three factors: T stage, GS and pre-treatment PSA [Zelefsky et al., 1998; Connel et al., 2001; Anderson et al., 1997]. Many researchers sub-stage these patients into three categories: Group I – Favorable; Group II – Intermediate Risk; Group III – High Risk [Zelefsky et al., 1998; Connel et al., 2001].

Options of Treatment for Non-metastatic Patients

Radiotherapy [RT] and radical prostatectomy [RP] are considered the treatments of choice for most patients with non-metastatic prostate cancer, with equal long-term outcomes [Abdalla et al., 2002]. Numbers Needed to Treat [NNT] calculations in a recent evidence-based study favors RT in terms of quantitative outcomes [Abdalla et al., 2002]. However, significant controversy exists as to the superiority of RT vs. RP, and a discussion on this issue is beyond the scope of this protocol. This study is for patients who have received RT.

The Extent of Biochemical Failure after RT

Between 30-50% of newly diagnosed non-metastatic prostate cancer patients undergo RT – either by their own choice or based on their physicians' recommendations [Savage et al., 1997; Yan et al., 2000; Shaw et al., 2000; Brandeis et al., 2000; Meltzer et al., 2001]. So, of the approximately 221,000 men diagnosed with prostate cancer, 90% [199,000] have non-metastatic cancer; of these, 66,300 [30%] to 110,500 [50%] will undergo radiotherapy. With a 30-50% rate of biochemical failure, as many as 33,000 to 55,000 patients will need an intervention that is now often a Total Androgen Blockade or Near-Total Androgen Blockade with LHRH-Agonist, which has a resultant loss of quality of life and significant cost.

Among these patients who undergo RT – either external beam or brachytherapy – between 30-40% will have PSA-based biochemical failure, mainly in those who had more than one or two advanced prognostic features among the three factors described above, viz, T3 or T4 stage, GS $\geq$ 6, or PSA $\geq$ 10 ng/ml [Zelefsky et al., 1998; Connel et al., 2001; Anderson et al., 1997; D'Amico et al., 2000; Shipley et al., 1999]. Recent evidence indicates that initial PSA values and [PSA-based] biochemical failure predict future clinical failure and prostate cancer-related death [Jani et al., 1999; Kupelian et al., 2002; Small et al., 2001; Palmberg et al., 1999].

From the above discussion, the following can be concluded:

- A significant percentage of 221,000 newly diagnosed patients undergo RT as their primary modality of treatment.
- Among these, a significant proportion carry poor prognostic features – individually or in combination – such as T3-4 disease, Gleason Sum of $\geq$ 6, and/or pretreatment PSA values of $\geq$ 10 ng/ml.
- These patients have higher chances of failure.
- The current intervention that is often used – the use of Androgen Blockade - significantly interferes with the quality of life and is quite expensive since these therapies are often continued for life and these patients have close to 85-90% 10-year survival rates [see Section 2.3 below].
- Post-treatment PSA levels can be used to detect early failures, and such PSA-based biochemical failures can be used to identify those patients who are likely to develop subsequent metastases.

THE MULTI-CENTRIC NATURE OF PROSTATE CANCER, "FIELD-CHANGES," AND PRE-MALIGNANT LESIONS

Prostate Cancer is a Multi-centric Disease

There is a general consensus that high-grade PIN lesions are precursors of subsequent development of prostate cancer [see for example: Sakr and Partin, 2001; Haggman, et al. 2000; Bostwick et al., 2000; Foster et al., 2000]. PIN is characterized by cellular proliferation within preexisting ducts and glands with cytological changes mimicking cancer. [Foster et al., 2000; Sakr et al., 2001; Qian J., 1998; Haggan et al., 1997].

The Reasons for Concluding that High-grade PIN Lesions are Likely Precursors of Subsequent Development of Prostate Cancer

- There is a significantly increased risk that patients with isolated high-grade PIN [HGPIN] will have prostate cancer confirmed on subsequent biopsy.
- HGPIN is found in association with cancer in 63% to 94% of malignant and 25% to 43% of benign prostates in autopsy studies.
- Data on age and race suggest that African-American men develop more extensive HGPIN at a younger age than white men. African-Americans have the highest incidence of prostate cancer in the world, about 1½ times higher than in U.S. whites. The mean age at diagnosis is also lower for African-Americans than for white Americans.
- A wide spectrum of molecular/genetic abnormalities appears to be common to both HGPIN and prostate cancer [for example: loss of 8p, 10q, 16q, 18q, and gain of 7q31, 8q, multiple copies of the c-myc genes, along with changes in chromatin texture, telomerase activity, etc.; Sakr and Partin, 2001; Foster et al., 2000].
- Cytogenetic links have been shown between high-grade pre-invasive neoplasia [PIN] lesions and prostate cancer [Alcaraz et al., 2001; Foster et al., 2000]. [For example: FISH analysis showing a high correlation (75% cases) in ploidy [aneuploidy] and pattern of cytogenetic alterations [trisomy 7, trisomy 8, and monosomy 8] between high-grade PIN areas and the paired prostate cancer focus in the same specimen [Alcaraz et al., 2001]. Similar findings are reported by Zitzelsberger et al. [2001].
- The incidence of PIN steadily increases with age of the general population, and African-American males have increased incidence of high-grade-PIN, which is highly correlated with increased incidence of prostate cancer [Powell et al., 2000].

The Causes of Failure Locally within the Prostate Gland after RT are not Well-established

There are two likely possibilities: (a) clonal growth of radio-resistant cells that survived the irradiation and/or (b) new development of malignant cells from normal or precancerous cells present in the prostate at the time of irradiation. It is not clear at this time what factors act upon the normal or precursor cells in the prostate in the process of malignant transformation; however, there is no reason to believe that whatever factors acted upon the prostate glandular cells in a patient prior to RT would change after RT. Thus, the prostate glandular cells left intact after RT are likely to become malignant once again. Consequently, chemopreventive agents that can stop

or delay the transformation process from normal and/or premalignant lesions to malignant lesions need to be studied.

TREATMENT FOR BIOCHEMICAL FAILURE AND THE DETERIORATION OF QUALITY OF LIFE

No standard treatment exists for the management of patients whose failure is detected based on PSA criteria. The current options include Androgen Ablation with LHRH-Agonists with or without Oral Anti-Androgens [Sylvester et al., 2001], Salvage RP for biopsy-documented local [prostate gland only] failure [Vaidya and Soloway, 2001], Intermittent Androgen Suppression [Crook et al., 1999], and observation alone.

All the interventions carry morbidities and losses of quality of life.

- Androgen Ablation is associated with hot flashes, loss of libido, inability to attain penile erection, tiredness, gynecomastia, and loss of bone mineral density.
- Intermittent Androgen Ablation carries the same complications as Androgen Ablation, except that, during the period when the patient is not receiving the LHRH-agonists, his side effects may subside.
- Salvage RP is rarely practiced and only a few Uro-Oncologists perform such procedures in a highly selected number of patients. A study conducted by CALGB in which the PI for the current study was a co-PI tested the feasibility of salvage prostatectomy. Fewer than five patients were accrued over three years. If performed, the chances of incontinence and impotence are higher than those associated with 'up-front RP' [i.e., those performed as first line of treatment at the time of diagnosis of prostate cancer].

For the above reasons, any intervention that can prevent or delay a biochemical failure is highly desirable.

VITAMIN D ANALOG - 1,25(OH)2D3 - AS A CHEMOPREVENTIVE AGENT

The role of vitamin D in cell proliferation and differentiation has been well established (Mehta and Mehta 2002, Miller 1999). Vitamin D and its analogs have shown laboratory and clinical evidence of chemoprevention and cytotoxic activity (Chen 2003; Guyton 2003; Krishnan 2003). The active metabolite of vitamin D 1α, 25-dihydroxyvitamin D3 suppresses cell proliferation of many cell types, including prostate cancer (Mehta and Mehta 2002, Boullion et al 1995, Campbell 1996). However, the use of 1,25(OH) 2D3 in clinical practice is limited due to its severe toxicity at a concentration required to suppress cell growth. Therefore, numerous analogs of vitamin D have been synthesized and evaluated for efficacy and toxicity in a variety of models. Of these several hundred analogs, EB1089, RO24-5531, 22-oxa-calcitriol, 25-hydroxyvitamin D3, and 1α-hydroxyvitamin D5 have been successfully used at relatively non-toxic concentrations in experimental in vivo carcinogenesis models and have progressed for evaluation in clinical trials. We synthesized $1\alpha(OH)D_5$ a few years ago (Mehta et al 1997) as an analog of the vitamin D5 series of compounds, since it was considered the least toxic in the series of vitamin D analogs (vitamin D2 to vitamin D7). As described later under the section, 'Preliminary Results,' we also showed that this analog could be tolerated at higher concentrations than any of the other efficacious analogs of vitamin D. It mediates its action via

vitamin D receptors, inhibits cell transformation, but does not affect normal breast epithelial cell growth.

Although the majority of the work with $1\alpha(OH)D_5$ has been done with breast cancer cells and mammary carcinogenesis models (Mehta RR 2000), we evaluated its efficacy in LNCaP prostate cancer cells. Results showed antiproliferative effects of $1\alpha(OH)D_5$ at 10^{-6}M concentration. In an in vivo study, LNCaP cells were inoculated in athymic mice and were treated either with vehicle or with 12.5 mcg/kg diet of $1\alpha(OH)D_5$ for 8 weeks. Tumor size was measured weekly. Results showed that $1\alpha(OH)D_5$ suppressed growth of LNCaP cells in athymic mice. These results indicate that $1\alpha(OH)D_5$ may be efficacious against prostate cancer in addition to its activities against the breast cancer. The effect of $1\alpha(OH)D_5$ in the experimental prostate carcinogenesis model has not been published. However, an experiment has recently been completed in our laboratories, where prostate cancer was induced in rats with MNU, and the animals were then treated with 50 mcg/kg $1\alpha(OH)D_5$-supplemented diet. Histopathological results from this study have not been evaluated (McCormick, Mehta, and Bosland: unpublished data), but soon will be available.

Prior to undergoing clinical evaluation, any compound has to be evaluated for safety and "dose finding" in two species under Good Laboratory Practice regulations. We recently completed a preclinical toxicity study under a subcontract to IIT Research Institute (Dr. McCormick) to determine dose tolerance in Beagle dogs and Sprague Dawley rats. These preclinical toxicity results are described under a separate heading in this document [See Section 4].

Following is a list of preliminary results generated in our laboratories rationalizing the selection of the agents and procedures for the current application.

- We had reported synthesis of $1\alpha(OH)D_5$ for initial studies, and since then it has been synthesized under good manufacturing practice (GMP) for Phase I clinical trials for breast cancer studies.

- $1\alpha(OH)D_5$ induces cell differentiation and inhibits cell proliferation of VDR+ breast cancer cells. *In vitro*, when breast cancer cells were exposed to $1\alpha(OH)D_5$ (0.1-10 μM), an antiproliferative effect was observed. *In vitro* treatment for 7-10 days also showed induction of various biomarkers associated with breast cell differentiation (such as α2 integrin, ICAM-1, nm23 lipid accumulation, and accumulation of β casein) in breast cancer cells positive for VDR. VDR-/+ MDA-MB-231 only marginally responds. Breast cells (only VDR+) exposed to $1\alpha(OH)D_5$ *in vitro* lost their tumorigenic ability when transplanted into mice.

- Prostate cancer cells sensitive to androgen, LNCaP cells, are VDR+ and respond to both 1α,25-dihydroxyvitamin D3 and $1\alpha(OH)D_5$, with a similar growth responsiveness as MCF-7 cells.

- LNCaP cells also exhibit induction of VDR following incubation for 7 days with 1 μM $1\alpha(OH)D_5$.

- Both 1α(OH)D_5 and 1α,25 $(OH)_2$ D_3 induced TGFß$_1$ in the alveolar cells of this tissue. 1α-Hydroxyvitamin D5 was effective against MNU-induced rat mammary carcinogenesis. It inhibited both incidence and multiplicity in Sprague-Dawley rats at 25 and 50 mcg/kg diet without any hypercalcemic activity.

- 1α(OH)D_5 at 12.5 mcg/kg diet inhibited growth of ZR75/A, T47-D, and BCA-4 cells in athymic mice. However, MDA-MB-231 cells did not respond to 1α(OH)D_5.

- 1α(OH)D_5 shows in vivo growth-inhibitory action on LNCaP prostate cancer cells. Preliminary studies show that 1α(OH)D_5 inhibits *in vivo* growth of prostate cancer cells. An in vivo experiment was performed on a small group (n = 4) of animals. Prostate cancer LNCaP cells were injected s.c. in 6- to 8-week-old male Balb/c athymic mice. Animals were given a control diet or a diet supplemented with 20 mcg/kg diet 1α(OH)D_5. Eight weeks after treatment initiation, only 1/4 (25%) of 1α(OH)D_5-treated animals showed tumor development; in controls, 4/4 (100%) animals showed tumor development. Mean tumor volume in 1α(OH)D_5-treated animals (n = 2 only developed tumor) was 0.06 cm^3 vs 0.15+0.05 cm^3 (n = 4) in control group. The PI realizes that our sample size is too small to determine statistical significance. However, the results shown here are preliminary in nature and suggest that 1α(OH)D_5 could serve as a potential therapeutic agent for prostate cancer cells.

- Preclinical toxicity was determined in rats and dogs. The rats received 28 days gavage treatment of increasing concentrations of 1α(OH)D_5 in a range of 2.5-10 mcg/kg body weight for CD-1 rats and 5-50 mcg/kg bodyweight for dogs. A complete battery of in-life, clinical pathology, and histopathology evaluations were performed. No toxicity or enhanced calcium levels were observed in rats. In beagle dogs, concentrations of 5 mcg/kg body weight resulted in no toxicity, whereas concentrations greater than 10 mcg/kg body weight resulted in loss of body weights, increased calcium, and gross toxicity. These results were utilized to develop a clinical Phase I trial protocol for breast cancer patients. We hope to be able to use these data for the proposed trial in this application. These maximum tolerated doses are considerably higher than 1α,25-dihydroxy D3 .

- 1α(OH)D_5 has the potential to advance from the laboratory to the clinic. 1α(OH)D_5 is scheduled to be used in a phase I clinical trial in breast cancer patients under a U.S. Army CTR breast cancer research award (# BC984013).

INTERMEDIATE BIOMARKERS IN PROSTATE CANCER

Selecting intermediate endpoint markers for the diagnosis, progression, or response to treatment for cancer patients has been a major challenge. In this respect, prostate cancer diagnosis has been considerably simplified by the examination of PIN and PSA. Numerous markers have been evaluated for a variety of chemopreventive agents for prostate cancer (Lazzaro, 2000). The intermediate biomarkers used for the two-cohorts in Phase II chemoprevention clinical trials include PIN (nuclear polymorphism, nucleolar size, and DNA ploidy), proliferation kinetics check points including PCNA, apoptosis, loss of heterozygosity,

and signal transduction markers including TGFα and β, IGF, c-erbB-2, and PSA levels. These markers have to be selected based on the progression of the disease as well as the chemopreventive agent. In a prostate cancer Phase II clinical trial with N-(4-hydroxyphenyl) retinamide, several additional markers were used, including p53, ploidy, and EGF receptors (Lazzaro, 2000).

RESEARCH METHODS

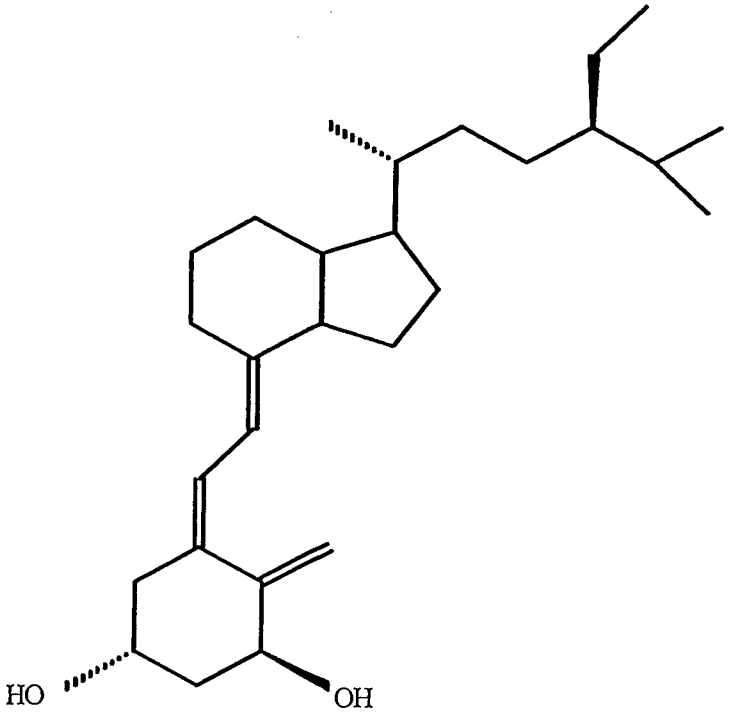
1α-HYDROXYVITAMIN D5

The analog 1α(OH)D_5 was synthesized by ConQuest, Inc. (Chicago, IL) under GMP (Good Manufacturing Practice) for the Phase I/II clinical trial for breast cancer patients and is available from Merrifield Pharma, Inc. (Westmont, IL) for the present study. We also have completed (as a subcontract to IIT Research Institute, Chicago) preclinical toxicity studies in two species. We will purchase 1α(OH)D_5 from Merrifield Pharma, Inc. (ConQuest, Inc. was sold to United Therapeutics in 2000 and no longer manufactures D5.) Meeting the prerequisites for using a compound in a clinical setting is very crucial for the success of the project. The current study therefore can be implemented clinically without any delay, once FDA approval is obtained.

Physical, Chemical and Pharmaceutical Properties and Formulation

Chemical Information

1α-Hydroxyvitamin D5 Structural Formula



Chemical Name: 1α-Hydroxyvitamin D5 is a structural analog of vitamin D5. The chemical name for it is 1α-Hydroxy-24-ethyl-cholecalciferol
[1α(OH)D_5]
Synthesis: The compound has been synthesized by Dr. Raju Penmasta, Merrifield Pharma, Inc., Westmont, IL, according to the Good Manufacturing Practice guidelines.
Molecular formula: C29H48O2
Molecular Weight: 428.6
Physical form: White powder
Solubility: It is insoluble in water but highly soluble in ethanol.
Purity: The acceptable limit for the purity of the substance is 95-100%, and the analytical method used to assure the identity and purity of the compound is reversed-phase HPLC. The compound, 1α-hydroxyvitamin D5, was separated on a C18-reversed phase 75x 4.6 mm, 3.5 micron column using a mobile phase of 90% acetonitrile in water. 1α-Hydroxyvitamin D5 was separated with a flow rate of 1 ml/min and monitored at 265 mμ. It was eluted with the retention time of 35 min.

PHARMACEUTICAL INFORMATION

The compound is being formulated in the form of an oral capsule. The concentration in each capsule will be created according to the protocol approved for the Phase I clinical trial. This will be comparable to the oral capsule given to animals in preclinical toxicity studies under Good Laboratory Practice guidelines. The capsules for each dose level will be prepared according to the dosage schedule at the time of the initiation of the study (Table 1). This will be prepared within the Pharmaceutical Science Department in the School of Pharmacy at the University of Illinois. All the inactive ingredients in the capsule will be standard pharmaceutical components, which comply with pharmacopeal guidelines. The capsules will be stored in a freezer to avoid degradation of vitamin D.

The control group will receive corn starch-filled capsules as placebo. These pills will not have any D5 in them. This is the same material that will be used for the control in the trial. The chemically-defined corn starch will be obtained from Colorado Sweet Gold, LLC, 8714 State Highway 60, Johnstown, Colorado 80534 (Contact: Charlie Gilbert at 970-587-6520).

PACKAGING AND LABELING THE STUDY DRUG

The blinded study medication will be delivered in containers by UI Chicago. Each container will contain medication for one week of treatment. The labeling on the medication will be in English and includes:

--medication is intended for clinical purposes only
--the name of the manufacturer: Merrifield Pharma
--the active ingredient
--the dosage per tablet (only for oral use)
--dosage instructions per day
--number of tablets per strip
--storage requirements

--expiry date
--packaging number
--name of the treating physician
--protocol number
--subject number
--treatment weeks
--this medication must be kept out of the reach of children
--yellow warning label with the remark: drug for clinical trial use only
The medication should be kept in the refrigerator.

PRECLINICAL TOXICITY

The main reason new analogs of vitamin D are being developed is to generate compounds with reduced or no toxicity. The analog $1\alpha(OH)D_5$ is one such relatively non-toxic vitamin D analog. We have completed an extensive series of preclinical toxicity studies for this vitamin D analog. In this section, we describe gross toxicity, calcemic activity in vitamin D deficient rats, and preclinical toxicity studies in two species, rats and dogs, under GLP.

GROSS TOXICITY

Treatment of animals with vitamin D analogs often results in loss of body weight. This is the first noticeable toxicity in animals. During the past few years, several experiments were performed where mice and rats were used as experimental models. As shown below, the tolerated doses for athymic mice, Balb/c mice, and Sprague Dawley rats were determined. These doses represent concentrations at which there was no loss of body weight or no adverse effects on general health. The animals were weighed twice per week and observed daily for lethargy and other noticeable changes.

MEASUREMENTS OF CALCEMIC ACTIVITY IN VITAMIN D-DEFICIENT RATS

Male rats three weeks of age were fed a diet containing 0.47g% calcium, 0.3g% phosphorus and no vitamin D. After three weeks of consumption of this diet, serum calcium levels were measured on selected animals. Animals exhibiting serum calcium values of less than 6.0 mg/dL were considered as vitamin D-deficient. The rats were treated with appropriate vitamin D analog for 14 days intragastrically. At the end of the study, the calcium concentrations were measured in the serum. The vehicle-treated control rats showed calcium concentrations of 5.4 ± 0.3 mg/dL (mean $\pm$ standard deviation). When animals were injected with 0.042 mcg/kg/day of vitamin D analogs, the plasma calcium concentrations of 6.0 ± 0.6 mg/dL for $1\alpha(OH)D_5$ (11% increase over control, statistically not significant from that of the control) and 8.1 ± 0.1 mg/dL for 1α,25(OH)D2 D3 (50% increase over control, statistically significant) were observed. At a higher concentration of 0.25 mcg/kg/day, $1\alpha(OH)D_5$ exhibited plasma calcium concentration of 8.1 ± 0.1 mg/dL as compared to 10.1 ± 1.8 for 1α25(OH)2 D3. Although both analogs increased serum calcium in comparison to the control samples, these results showed overall lower calcemic effects for $1\alpha(OH)D_5$ as compared to 1α,25(OH)2D3.

Experiments were carried out to determine maximum tolerated dietary dose of 1α(OH)D_5 for rats. Sprague-Dawley rats were separated into 11 groups of 10 animals each. Group 1 served as a control. Rats in other groups received either five doses (0.8, 1.6, 3.2, 6.4, and 12.8 g/kg) of 1,25(OH)2D3 or five doses (3.2, 6.4, 12.5, 25, and 50 g/kg) of 1(OH)D_5 for six weeks. Results showed that there was hypercalcemia and loss of body weight observed at 12.8 g/kg diet, whereas there was in fact increased body weight observed at 50 g/kg of 1α(OH)D_5 dose level. In a separate study, there was no adverse effect of D5 on the body weight gain observed at 100 g/kg diet. Therefore, the 1(OH)D_5 can be tolerated at a much higher concentration than the dihydroxy-D3 analog of vitamin D.

Preclinical Toxicity (GLP)

Four-week oral (gavage) toxicity studies were performed on rats and dogs at the IIT Research Institute in accordance with the U.S. Food and Drug Administration (FDA) Good Laboratory Practice (GLP) regulations as set forth in the *Code of Federal Regulations (21 CFR Part 58).*

Studies in Rats

A 28-day toxicity study was performed in both male and female CD rats. Ten animals per sex per dose were entered in the study. 1α-Hydroxyvitamin D5 was administered in corn oil at three dose levels: 2.5, 5.0, and 10 mcg/kg of body weight. A control group of rats received only vehicle. Ten additional animals were kept in control and high dose groups for a 14-day recovery period. All animals were observed for adverse clinical signs, body weight gain, and food consumption. Clinical pathology, hematology, and clinical chemistry measurements were carried out for every animal. All animals were subjected to gross necropsy, and tissues from control and high-dose animals were processed for histopathological evaluation. No animals died from the treatment during the study. No clinical signs or adverse toxicity-related symptoms were observed at any dose level. No effect on food consumption or body weight gain was observed during the study. Treatment-related increased calcium was observed in the high-dose group (Control 11.0 $\pm$ 0.46 vs. high-dose 11.6 $\pm$ 0.73 mg/dL). Calcium and phosphorus were not increased in the recovery group of animals. Increased incidence of mineralization in the kidneys was observed at high doses. All microscopic changes were of minimal to mild severity. In summary, there was a minimal severity of mineralization observed in kidneys at high-dose level in both sexes. These lesions often occur as incidental findings in rodent studies. Therefore, although an absolute no-effect level dose was not established, minimal toxicity was observed in these experiments and that might not be 1α-hydroxyvitamin D5-related .

Four-Week Oral Toxicity Study in Beagle Dogs

A 28-day oral toxicity study was performed in both sexes of beagle dogs to evaluate the toxic effects of 1α-hydroxyvitamin D5. The vitamin D analog was administered in a vehicle of corn oil in 1 ml volume /kg/day at three dose levels of 10, 30, and 90 mcg/kg/day. The vehicle was administered in the control group of dogs. Three dogs per sex per each concentration were entered in the study. Two additional dogs were kept for vehicle and high-dose group for a recovery experiment. However, because of mortality in high dose groups, the 2 dogs in the high dose recovery group were transferred to the toxicity study, and the 90 mcg/kg dose level was

reduced to 45 mcg/kg/day for the remainder of the study. The two dogs from the recovery group of the control group were dosed 5 mcg/kg/day for 28 days. Toxicological endpoints included physical examination, clinical observations, ophthalmic examination, body weights, food consumption, hematology, clinical chemistry, electrocardiographic evaluations, and histopathological evaluations for all animals. Eight dogs died during the study: 2 females and 3 males at 90 mcg/kg/day dose, and 2 males and 1 female at 30 mcg/kg/day. Toxicity was observed at all concentrations above 10 mcg/kg/day. Serum calcium increased at concentrations of 10 mcg/kg and above. However, no ophthalmic or cardiac toxicity was observed at any dose level. In summary, the results indicated that dogs were more sensitive to 1α-hydroxyvitamin D5 as compared to rats, and the maximum tolerated dose for this analog in dogs was 5 mcg/kg/day or slightly higher but less than 10 mcg/kg/day.

Summary:

Results described in this section have clearly defined the maximum tolerated dose levels of $1\alpha(OH)D_5$ and compared it to the 'standard' active metabolite of vitamin D (1,25-dihydroxyvitamin D3). Results showed that the D5 analog could be tolerated at more than 10 times the concentration of 1,25 dihydroxy D3 without affecting body weight or hypercalcemic condition. The preclinical toxicity in two species is completed under GLP regulations and results have indicated that it is safe to evaluate $1\alpha(OH)D_5$ for Phase I/II clinical trials.

PROCEDURES

FOLLOW-UP

1. Forty patients will be seen once every four months in the clinic, except in the initial 1-4 months as detailed in the box below. In 2003, the UC Davis Cancer Center saw 41 potentially eligible patients. In 2002, 28 potentially eligible patients were seen. Since investigators will draw on four years during which patients are eligible for the study (12-60 months post-radiation therapy), there will be a large enough pool of patients from which to draw subjects for this study (about 140 patients who meet study inclusion and exclusion criteria). Generally, prostate cancer patients are seen every four months after they complete radiotherapy, so this part of the study schedule poses no additional burden on the patients.
2. Patients will have blood drawn for PSA prior to digital rectal examination (DRE).
3. Patients will have a complete history taken, and a physical examination and a DRE performed.
4. Patients' compliance will be documented [Pill Diary].
5. Pill Diary will be submitted by patients.
6. Patients' symptoms [if any] will be documented.
7. Quality of Life forms will be completed. The Health Survey SF-36V forms will be used, as they have been validated [see attachment] (Ware 1995; Ware 1994).
8. The American Urological Association (AUA) GU Symptom Scoring Scale form (Appendix VI), which has been validated (Barry 1992), will be used in every follow-up appointment and will indicate whether, upon completion of radiation therapy, cancer

progression might be occurring. (This is a standard follow-up procedure for prostate cancer patients, not particular to this study.)

After informed consent is obtained, all 40 subjects will participate in a one-month run-in period, during which they will take one placebo pill per day. The investigators will look at the pill calendar that the patients have filled out, and count the number of pills left in the bottle at the end of the month to measure compliance; any subject who is not within 10% of the expected count will be considered non-compliant and will be withdrawn from the study. Since there are 28-31 days in a month, the 10% non-compliance threshold allows patients to miss at most three pills; if they miss four pills, they will not be able to participate in the study. (See Informed Consent form.)

INTENSIVE FOLLOW-UP SCHEMA FOR THE FIRST PHASE TO IDENTIFY ANY UNUSUAL 'REACTORS'

- During the first month of the study, patients will be seen once a week and an interview for any toxicity will be done by the CRA and blood will be drawn for calcium levels.
- If any symptoms develop, they will be seen by a physician
- If calcium levels are elevated x 1.5 times the base line, a dose reduction to 50% of the dose will be done
- If calcium levels are stable in the first month, then patients will be seen once a month; a telephone call will be made once a week from month 2 to 4
- If calcium levels are stable during the first 4 months and if the patients are clinically stable without any toxicity, then they will be seen once in four months; once a month phone calls will be made during the second 4-month period.
- Phone call evaluations will be discontinued if patients are clinically and biochemically stable for the first 8 months.
- Weekly evaluations of calcium and phosphorus in blood, albumin, Chem 7, and urine elctrolytes (urine samples will be collected from subjects).
- PTH at baseline and once every four months.

The collection of blood and urine samples will be done at the UC Davis Cancer Center and its affiliated facilities; standard precautions will be used. Plasma will be separated from the blood by centrifugation and saved at -20C. Similarly, urine samples will be saved at -20C prior to sending them to the University of Illinois (UIC). All Samples will be labeled with the appropriate human subject identification number without disclosing any additional information. Samples will be sent to the University of Illinois at Chicago (UIC) on dry ice for analysis. At UIC the samples will be stored at -20C in the freezer. Blood and urine samples will be used only for the vitamin D5 study analysis as described in the protocol; no other tests will be done on the samples without Human Subjects permission and Informed Consent. Samples for all subjects will be disposed of only after the study findings are analyzed and the study is closed. The studies will be carried out in the Department of Surgical Oncology research laboratories located at 840 South Wood Street, Chicago Illinois 60612. Dr. Rajendra Mehta, Co-Investigator for the project, will be in charge of these studies.

REGULAR FOLLOW-UP VISITS

After the Initial Intensive Follow-up, patients will be seen once in four months, or earlier if necessary. These visits will be exactly the same as the first 4-month visit, which included a physical examination, a digital rectal examination, blood tests [about 13 cc's drawn from the patient's vein] and filling in some forms. These forms ask questions about the patient's quality of life; that is, whether there are any changes in his abilities or enjoyment. These are the same forms study subjects were asked to complete at the beginning of the study. Our Clinical Research Associate will help them to complete the forms if the patients have any questions.

Summary of Schedule for Study Participants (40 patients)								
Month(s):	Run-in		1		2-4		5, 9, 13, 17, 21	24
Event/Procedure Week(s):	**1**	**2-5**	**1**	**2-5**	**1**	**2-5**	**1**	**5**
Informed Consent Given	X							
Clinic Visit	X	X	X	X	X		X	X
Physical exam by doctor	X						X	X
Digital Rectal Exam (DRE)	X						X	X
Complete History	X							
Lab Collection[1]	X	X					X	X
Clinical Research Associate (CRA) Interview	X	X	X	X	X		X	X
Quality of Life forms completed	X		X		X		X	X
AUA GU Symptom Scoring Scale completed	X				X		X	X
Karnofsky Performance Scale completed	X	X	X		X		X	X
Pill Calendar given	X		X		X		X	X
Placebo pills given	X							
Placebo pills taken daily	X	X						
Pill Calendar collected			X		X		X	X
Study pills given			X		X		X	X
Study pills taken daily			X	X	X	X	X	
Patient symptoms documented			X		X		X	X
PTH			X				X	
Biopsy (following initial biopsy at time of diagnosis)								X
Telephone Call by CRA				X	X	X		

[1] for PSA, calcium, phosphorus, albumin, Chem 7, and urine electrolytes

The end-of-study biopsies will be performed by UC Davis urologists. The tissue will be safely stored in the UC Davis Pathology Department.

WITHDRAWAL/TERMINATION FROM THE STUDY

A study subject may withdraw from the study at any time. Subjects should inform the Principal Investigator about their withdrawal from the study. Their participation is completely voluntary. Their decision to no longer participate will not affect their current or future relations with their doctors or other health care providers in the university. The Clinical Research Associates will

try to follow-up with those participants who decide to withdraw on the same schedule as those participating in the study. If the patients refuse to participate in this way, then the Clinical Research Associates will try to have phone contact with the former study participants.

Once a patient has withdrawn from a study, the Principal Investigator and other study members are no longer allowed to obtain any new information from the patient's medical records. They may continue to use patient information which was collected before the patient withdrew. Charts of patients who have withdrawn would be stored and maintained offsite (not kept with the active study charts), and the charts would be designated as "withdrawn".

There are a few circumstances under which the principal investigator would terminate a subject's participation in the study (other than serious side effects to the study medication). First, if the patient was non-compliant in taking the study medication, the Principal Investigator could terminate the subject's participation. Secondly, if the study subject is experiencing other serious medical conditions that would interfere with satisfactory continuation of the study, the Principal Investigator could terminate their further participation. As with the case when a subject chooses to withdraw, the Clinical Research Associates will try to follow-up with these participants on the same schedule as those participating in the study. If the patients refuse or are unable to participate in this way, then the Clinical Research Associates will try to have phone contact with them.

TREATMENT PLAN

Administration

The study medication will be dispensed monthly by the research nurse. All patients will receive a one-month supply of either D5 or the placebo at their monthly visit with the research nurse, along with the pill diary form to record their medication use. Both of the study arms will follow the same schedule of drug administration. The standard dose of D5 will be 10 mcg per capsule, taken once a day.

At each follow-up visit, an assessment of patient medication compliance will be made and recorded in the patient's medical record. Compliance will be recorded as the percentage of pills taken. To help in the assessment of compliance, it is required that patients keep a pill diary record (using the form provided to them) of their daily pill consumption. Prior to starting treatment, the patient will be provided with and instructed in the proper use of a pill diary (see Appendix XIII for this form). The patient will be instructed to return this diary at specified intervals during treatment and at each follow-up visit. This record will be checked for compliance by the investigator. The diary will be retained in the patient's record. The diary will act as source documentation. Patients who are non-compliant with diary use will be re-instructed in the use of the diary.

Discontinuation of Drug

Upon completion or discontinuation of D5 or placebo, the patient will be instructed to return all unused supply to the investigator for proper disposal.

Toxicity-Based Dose Modification Schedule for D5

Toxicity	Grade 3 or 4
1st appearance	The patient will go on a drug holiday for one month or until the toxicity has been resolved to grade 0-1, whichever is longer, then continue at 50% of starting dose (i.e., 5 mcg per day)
2nd appearance	Interrupt for one month or until resolved to grade 0-1, whichever is longer, then continue at 50% of previous dose (i.e., 2.5 mcg per day)
3rd appearance	Interrupt for one month or until resolved to grade 0-1, whichever is longer, then continue at 50% of previous dose (i.e., 1.25 mcg per day)
4th appearance	Discontinue treatment permanently

Identifying Patients Who Develop Emotional Problems

If the regularly administered Quality of Life assessment on any study subject suggests any significant deterioration, including psychological status, the same de-escalation protocol will be followed as is done for patients who develop medical toxicity to D5: drug holiday for one month, followed by a 50% reduction in D5 dose, up to three times.

MONITORING OF STUDY

The study will be conducted according to Good Clinical Practice (GCP) guidelines. GCP is a standard for the design, conduct, performance, monitoring, auditing, recording, analysis, and reporting of clinical trials. The Good Clinical Practice Program is the focal point within FDA for Good Clinical Practice issues arising in human research trials regulated by FDA.

Representatives of the U.S. Army Medical Research and Materiel Command (USAMRMC), the agency sponsoring this research study, and Department of Defense (DOD) may inspect the research records for this study at any time as a part of their responsibility to protect human subjects in research. Per HSRRB requirements, a medical monitor is assigned to this study.

DATA SAFETY MONITORING BOARD

The UCD Data Safety and Monitoring Committee will review the data at least every six months and evaluate the results.

ACCOUNTABILITY PROCESS FOR THE STUDY DRUG

The UC Davis Cancer Center has an Investigational Drug Service in its Pharmacy Department, headed by Victoria Bradley, Pharm.D. Investigational drugs, such as D5 for this study, are first sent directly to the Investigational Drug Service, and then they manage the distribution of the drug to the Cancer Center Pharmacy. Study coordinators must fax a patient's consent form to the

Pharmacy in order to receive the study drug. Files are regularly audited by the UC Davis Cancer Center Data Safety Monitoring Committee. The Pharmacy has a log system in place to keep track of all investigational drugs, which includes their receipt, storage, inventory, disposition, and the disposal of unused supplies.

ENDPOINTS OF THE STUDY

1. Proportion of Patients Having Rising PSA
   (Three consecutive increases in PSA; ASTRO criteria, Shipley et al., 1999).

2. Proportion of Patients Having PSA Failure (and using other definitions, such as doubling time)

   Definition of PSA failure is per Jani et al., *Urology*, 1999. Briefly, this definition derives from the observation that the logarithm of the PSA profile curve provides more applicable information about the natural history of failure than the PSA profile curve itself. This biochemical failure criterion is based on a quadratic curve fitting of the logarithm of the PSA profile. First, the logarithms of the follow-up PSAs are computed, and a quadratic curve, fPSA, is fitted through this log PSA profile. Biochemical failure is declared when the fPSA is twice the fitted nadir. Since normal PSA values are not indicative of failure, if the fitted nadir PSA is 1 or less, biochemical failure is declared when fPSA=2.

3. Proportion of Patients with Cancer Present in End of Study Biopsy Specimens

4. Toxicity

5. Number of patients for whom drug discontinuation or dose reduction is required; median number of days on full dose of drug.

6. Quality of Life

   See Appendix V for the Quality of Life form (6 pages). This will be administered every four months, during the regularly scheduled clinic visit.

7. Differences in Biomarkers Profile

Note: Patients on this study will continue to be followed beyond 2 years – as part of their regular cancer care, they will be followed until death or until study investigators lose contact with them. Only monitoring of toxicity and the end-of-study biopsy will cease after the 2 years of the study. Therefore, the clinical endpoint is indefinite.

STATISTICAL ANALYSIS

OVERVIEW

The primary aims of this study are to provide preliminary estimates of efficacy compared to placebo for design of a Phase III trial, and to assess the tolerability and safety of the vitamin D5 preparation. The study will be a randomized, double-blind intervention; randomization will use a permuted block design, stratified by baseline PSA level.

Analysts are blinded to treatment (Vitamin D or placebo). All specimens are identified only by a sample number, and the link between individual samples and subject ID is handled by our data management group using a firewall-protected server and locked files. Thus the analysts may be able to guess that an individual sample was drawn from a person receiving Vitamin D, but would be unable to link that sample to a specific subject ID or to the sample used to assess the outcome, PSA level.

Patients and investigators will be blinded to treatment assignment. It is possible that an investigator would be able to guess the treatment based on lab results, for example, an elevation in calcium level sufficient to require a dose reduction. We anticipate that such changes will be infrequent, based on pilot data. Since the PSA outcome will be measured objectively, the biggest risk to the integrity of our study will be if there is differential drop-out because of inadvertent unblinding by other lab results. Our Data and Safety Monitoring Committee (DSMC) will monitor treatment-specific changes in dosage and drop-out rates in a closed session. If the drop-out rate is low, the potential impact is small, and we can assess it directly by sensitivity analyses considering the potential effect of those who dropped out on the final results. If the drop-out rate is higher and appears to be differential, we will consider alternative analyses along the lines suggested by Robins and Rotnitzky, who have developed procedures for counterfactual analyses in clinical trials to take account of differential participation and non-compliance.

All analyses of clinical outcome will be intent-to-treat, while analyses of changes in marker values and toxicity will take account of treatment actually received.

Analysis of Primary Endpoints

The proportion of patients having rising PSA (both ASTRO and Jani definitions) will be summarized separately for the patients receiving D5 and placebo, and compared using Fisher's exact test. The proportion with cancer present in End of Study biopsy specimens will be compared similarly. Efficacy analyses will be intent-to-treat, and one-sided hypothesis tests will be used at level 0.05.

The proportion of patients experiencing toxicity and a 95% confidence interval will be calculated separately for patients receiving D5 and placebo, and compared using Fisher's exact test. The proportion for whom drug discontinuation or dose reduction was required will be summarized and compared similarly. Median number of days taking the full dose of the drug will be compared using non-parametric tests (Wilcoxon rank sum if no censoring, log rank if censoring.)

Analysis of Secondary Endpoints

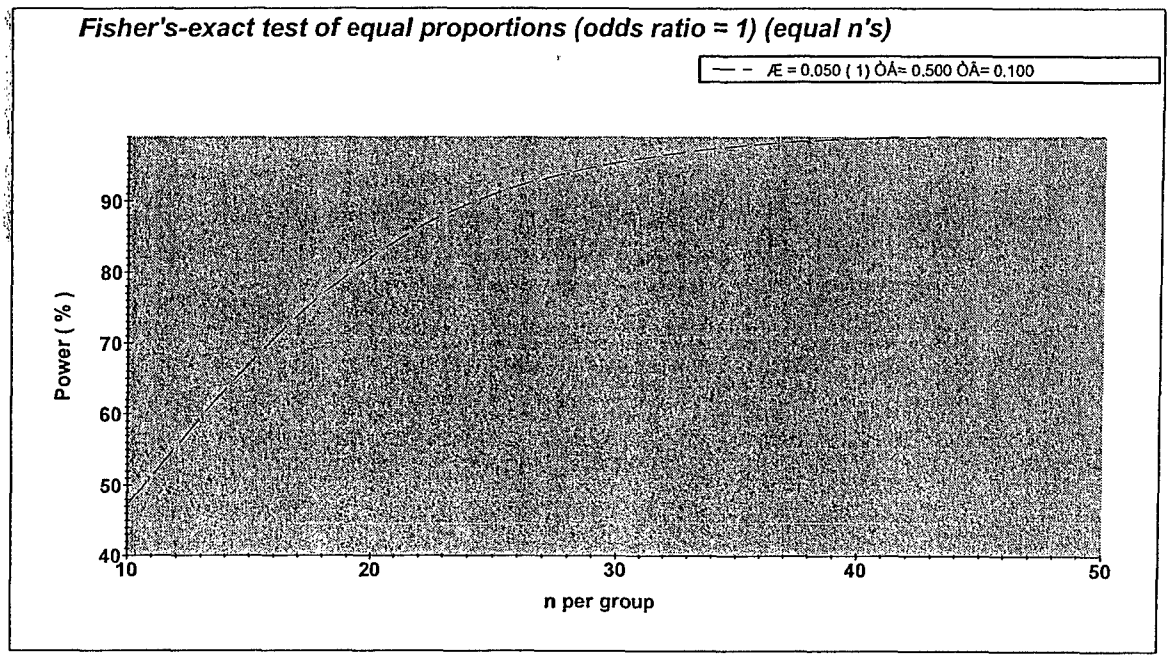
Both quality of life data and biomarker (PSA) data will be assessed every four months. Repeated measures regression models for longitudinal data (Laird and Ware, 1982) will be used to

summarize the patterns of change in quality of life score and in biomarker measurement over the study period. The difference between overall mean level on treatment and the average rate of change per month will be estimated and compared for patients on Vitamin D5 vs. those on placebo. These models allow for the use of all available data, even if some measurements are missing, and they allow for differences between individuals in baseline levels and rates of change, as well as within-person variation.

SAMPLE SIZE AND POWER CONSIDERATIONS

A sample size of 20 patients will be randomized to each group. The primary outcome will be recurrence of cancer, compared by one-sided Fisher's exact test at level 0.05. A one-sided test is appropriate because we will only consider a Phase III trial if there is evidence of efficacy. The proposed test will have 80% power to detect an improvement from a 50% recurrence rate with placebo (based on the literature) to a 10% rate with vitamin D5.

A sample size of 20 patients would ensure that we would observe, with 80% probability, at least one occurrence of any toxicity that occurred in at least 8% of patients, and with 90% probability any toxicity occurring in 11% or more of patients. We will be able to estimate the proportion requiring a dose reduction to at worst plus or minus 22% (based on 95% confidence interval and half of patients having difficulty tolerating dosage.) [Laird et al, 1982]



THE RATIONALE FOR THE 1α(OH)D5 DOSE IN OUR STUDY

TOXICITY OF CHEMOTHERAPEUTIC AGENTS

One pre-requisite in testing a chemotherapeutic agent in clinical studies is to conduct experiments in animal models to ascertain that the agent is effective at a *non-toxic concentration*

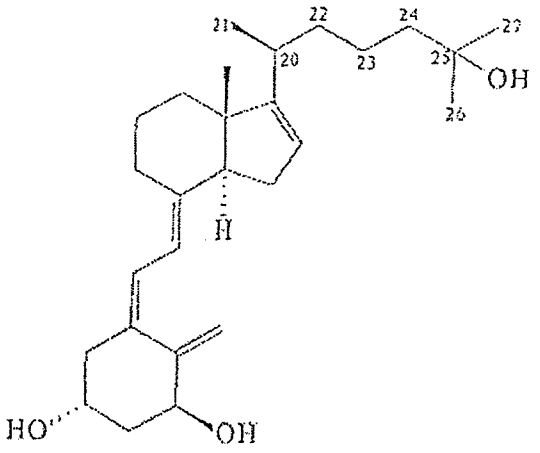
(Mehta and Mehta, 2002). One primary side effect of vitamin D is hypercalcemia. Therefore, any analog of Vitamin D has to be shown to be active at non-hypercalcemic concentrations, or, even if it causes hypercalcemia, such an effect should be shown to be minimal. It is also important to mention that some analogs may be non-calcemic, yet may not be tolerated at high concentrations, due to other toxicities. Therefore, in such cases, it is necessary to monitor the toxicity of the agent in a dose-response study.

This is usually achieved by establishing a maximum tolerated dose [MTD] for each chemopreventive analog of the potential agent – in this case vitamin D. So far, some of the analogs of Vitamin D have been evaluated in vivo for their efficacy in chemoprevention. These include:

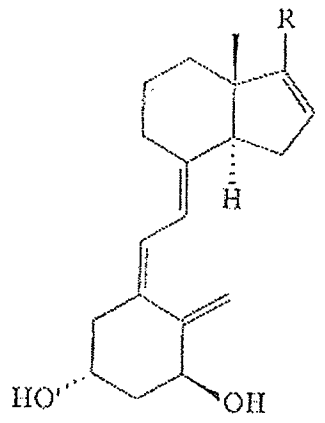
- RO24-5531 (Hoffman-LaRoche)
- EB 1089
- CB 966, MC903 (Leo Pharmaceuticals)
- 22-oxa-calcitriol (Chugai Pharmaceuticals Japan) and
- $1\alpha(OH)D_5$ (OncQuest Inc.)

CHEMICAL STRUCTURES OF ANALOGS (FIGURE 1 ON THE NEXT PAGE)

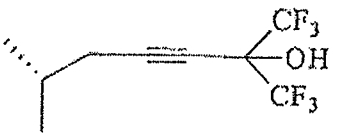
FIGURE 1



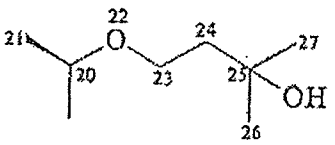
$1\alpha,25(OH)_2D_3$



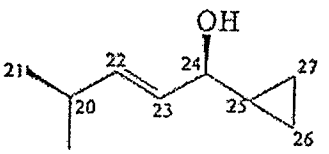
1. RO24-5531 R =



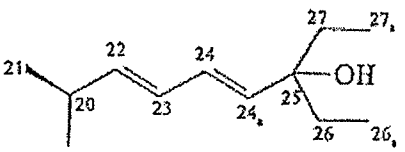
2. 22-Oxacalcitriol (OCT) R =



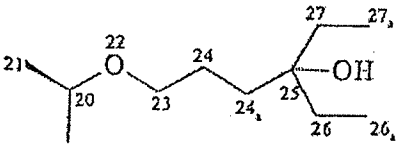
3. Calcipotriol (MC903) R =



4. EB1089 R =



5. KH1060 R =



6. 1α-Hydroxyvitamin D_5 R =

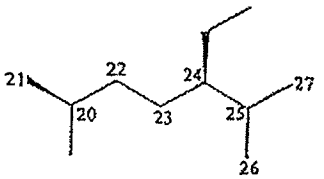


Fig. 3. Chemical structures of some of the active analogs of vitamin D.

EFFECTS OF VITAMIN D ANALOGS

The effects of vitamin D analogs have been studied mainly in mammary and colon carcinogenesis models to date. These results are summarized in Table 1.

Table 1: Summary of Efficacy of Vitamin D Analogs in Cancer Cell Proliferation (from Mehta RG and Mehta RR, 2002)

Table 1
Summary of efficacy of vitamin D analogs in cancer cell prolifration

Target organ	Cells	Vitamin D analogs	Efficacy	Comments
Breast	ER+			
	MCF-7, ZR75-1, T47D BT474, BT20, SK-BR-3	22-oxa-calcitriol, $1\alpha(OH)D_5$EB-1089, KH1060, MC903, RO24-5531, 22-oxa-Calcitriol	All effective	VDR+
	ER-			
	MDA-MB-231, MDA-MB-436	1α-$(OH)D_5$, 22-oxa-calcitriol, KH1060, RO24-5531	Ineffective	VDR+/-
	UISO-BCA-4	$1\alpha(OH)D_5$	Effective	VDR+
	UISO-BCA-1	1α-$(OH)D_5$	Ineffective	VDR-
	MDA-MB-231,	22-oxa-calcitriol	Effective	VDR+/-
Prostate	LnCap, PC-3	$1\alpha(OH)D_5$, EB1089, RO24-2637, 22-oxa-calcitriol, MC903	All Effective	VDR+
	Du-145	$1.25(OH)_2D_3$, RO23-7553	Ineffective	VDR+/-
	Du-145	RO24-5531, RO26-2198	Effective	
Colon	HT-29, CaCo-2	$1.25(OH)_2D_3$, RO24-5531	Effective	VDR+

IN VIVO EFFECTS ON PROSTATE METASTATIC MODELS

There are at least two reports that establish the role of vitamin D analogs in preventing or retarding the metastasis of cancer cells to a distant organ as described below and thus clearly hint that these selective analogs may be very influential against the cancer cell metastasis:

1. Effects of 1,25-dihydroxyvitamin D3 was evaluated and compared with EB1089 in transplantable prostate tumor model using androgen-insensitive metastatic rat prostate model. MAT LyLu cells were injected in Copenhagen rats and appropriate groups were treated with low (0.5 mcg/kg) and high (1 mcg/kg) doses. Both these analogs reduced the metastatic foci in lungs in these rats. However, this benefit was accompanied by hypercalcemia and loss of body weight at higher dose.

2. More recently, we evaluated effects of $1\alpha(OH)$ D_5 on the growth of LNCaP cells in athymic mice (unpublished) in our laboratories. Results showed that 55 nmole/kg (25 mcg/kg) of the vitamin D analog D5 in the diet for 60 days resulted in reduced tumor volume as compared to the control LNCaP tumors. At 55 nmole/kg diet concentrations, the D5 analog did not elevate serum calcium levels. Thus, this experimental evidence indicates not only that these vitamin D analogs [D3 and D5] are effective as

chemopreventive agents in experimental [prostate] carcinogenesis models but also that they suppress the growth of human cancer cells in athymic mice [i.e., Cytostatic].

IN VIVO EFFECTS ON PROSTATE NON-METASTATIC MODELS

There are two studies conducted with $1\alpha(OH)D_5$ in prostate non-metastatic models. One study is carried out in rats. In this model, prostate cancers are induced by MNU and treated with dietary modulation of 50 mcg/kg of $1\alpha(OH)D_5$ for a two-year period. This study is just completed, awaiting histopathological evaluations (McCormick, Mehta, and Bosland in progress). A summary of these results is shown in Table 2.

Table 2: Effects of Vitamin D Analogs on Different Carcinogenic Models of Target Organs (from Mehta RG and Mehta RR, 2002)

Table 2
Summary of efficacy of vitamin D analogs in chemical carcinogenesis models

Organ	Models	Analog	Dose	Efficacy	Comments
Breast	MNU-induced adenocarcinoma	RO24-5531,	1,10 nmole/kg diet	Effective	No toxicity
		1α-Hydroxyvitamin D_5	58.4, 116.8 nmole/kg	Effective	No hypercalcemia
				Dose related effect	No loss of body weight
		1α-hydroxy D_3	0.25 nmole	growth inhibition	Treatment schedule
		$1,25(OH)_2D_3$	0.59-2.99 nmole/kg	No Effect	Hypercalcemia
		MC903	111 nmole/kg	Growth inhibition	Hypercalcemia
		EB1089	1.1-5.5 nmole/kg	Effective	Hypercalcemia
					Loss of body weight
Prostate	MNU-induced	RO24-5531	10 nmole/kg	Effective	No toxicity
					No effect on dorsal prostate
		$1\alpha(OH)D_5$	58.4-116.8 nmol/kg diet	In progress	
Colon	AOM-induced	RO24-5531	2.5 nmole/kg ip	Effective	No toxicity
	DMH-induced	22-oxa-Calcitriol	72.5 nmole/kg ip	Effective	
	DMH-induced	24R,25 dihydroxyvitamin D_3	0-24 nmole/kg	Effective	Reduced aberrant crypt
	DMH, MNU, and nitrosamines	24R,25 dihydroxyvitamin D_3	0-12 nmole/kg	Effective	foci colon only

Below, we have tabulated the MTD doses that have been established from animal studies and the type of toxicity for various Vitamin D Analogs:

Table 3: Maximum Tolerated Dose (MTD) Ranking for Commonly Used Vitamin D Aanalogs in Experimental Animals

Vitamin D Analog	Maximum Tolerated Dose (MTD)	Toxicity
1α Hydroxyvitamin D5	116.8 nmole/kg diet	None
MC903	111 nmole/kg diet	Hypercalcemia
22-Oxacalcitriol	72.5 nmole/kg BW ip	None
24R,25 Dihydroxyvitamin D3	24 nmole/Kg BW	None
RO24-5531	10 nmole/kg diet	None
EB 1089	5.5 nmole/kg BW	Hypercalcemia
1,25 Dihydroxyvitamin D3	2.99 nmole/kg BW	Hypercalcemia
1α Hydroxyvitamin D3	0.25 nmole/kg BW	None

It can be seen that D5's MTD is higher than all other analogs; also even at very high doses, no evidence of hypercalcemia has been demonstrated.

The above results show that:

1. $1\alpha(OH)D_5$ is relatively well tolerated at much higher doses in experimental models than the doses of 10 mcg per day that is being planned in this clinical trial.
2. This dose is unlikely to pose any major clinical toxicity with long-term use, although the clinical trial is designed to carefully monitor the patients for any unexpected toxicity and to initiate either stoppage or dose reduction, if such unexpected toxicities occur.
3. As has been shown for 1,25(OH)2D3 [which has successfully reduced the rate of elevation of PSA in prostate cancer patients], it is reasonable to expect $1\alpha(OH)D_5$ to be just as effective, but with fewer or no drug-related side effects/toxicities.

Finally, the Principal Investigator for this study, Dr. Srinivasan Vijayakumar, has previous experience with Phase II clinical trials (Vijayakumar 1993; Sweeney 1998).

2. SUBJECT SELECTION

ELIGIBILITY CRITERIA

1. Men who had received radiotherapy with curative intent. These patients should have had non-metastatic prostate cancer, i.e., no clinical or imaging evidence of distant metastases or lymph-nodal metastases. They should have been staged by standard procedures:

    - Digital Rectal Examination and documentation of the pre-RT findings in a AJCC Staging Sheet
    - Pre-treatment biopsy and a report of the grade of the lesion

- Pre treatment PSA levels (must be between 2 and 8 at the time of registration)
- Bone Scan is recommended if the PSA level is over 15 ng/ml at the time of diagnosis [Chybowski et al., 1991; Vijayakumar et al., 1994]

2. The radiotherapy:
   - Should have been completed within 5 years from the date of registration, but not within the immediate twelve months [see below]. Study entry criteria is based on clinical and biochemical status, so enrolling patients at different time periods after treatment will not cause a problem.
   - Could have been external beam RT [XRT] alone, XRT with neoadjuvant hormonal therapy of brief duration [not exceeding 12 months], brachytherapy alone, brachytherapy with neoadjuvant hormonal therapy of brief duration [not exceeding 12 months], or a combination of XRT and brachytherapy [again, if neoadjuvant hormonal therapy was given, it should have been for a duration not exceeding 12 months]

3. There should have been no evidence of metastatic disease at the time of diagnosis.

4. There should be no evidence of metastatic disease at the time of registration.

5. The PSA should have been stable [no more than 0.75 ng/ml variation in the PSA measurements], with at least 3 measurements within 12 months prior to the date of registration.

6. The Karnofsky Performance Status [KPS] should be 80% or more.

7. Patients have to sign an informed consent. They should be able to understand and consent in a fully informed document.

8. They should belong to <u>Group II or III</u> based on T-stage, Gleason Sum and PSA criteria:
   - Group I = T1/T2 AND Gleason Sum <6 AND PSA < 10 ng/ml
   - Group II = One of the three factors higher than under Group I
   - Group III = Two or more of the three factors higher than under Group I

9. The age range of the subjects will be from 18 to 65+ years of age. There will be no maximum age limit for study subjects (Hall et al., 2004).

10. There are no medications and/or treatments, other than those listed in the inclusion/exclusion criteria, which study subjects must avoid due to the study medication.

ANONYMITY OF STUDY SUBJECTS

The anonymity of the study subjects will be maintained. In study records, subject names will not be used. Only initials will be used. No social security numbers will be used. Study coordinators will maintain a tracking book and be given a case study number for each study subject. *No identifiers, used for recruitment purposes, will be disclosed to a third party except as required by law or for authorized oversight of the research project.*

Any study records are going to be kept in a secure, locked cabinet in the Clinical Trials office. All of the University's data is password protected and only employees associated with the study will have access to them. Per University policy, study records will be maintained for 10 years.

EXCLUSION CRITERIA

1. Patients with metastatic disease.
2. Patients with a rising PSA as defined by the American Society for Therapeutic Radiology (ASTRO) criteria of three consecutive increases in PSA. PSA doubling time must be ≤ 6 months.
3. Patients who are on Androgen Deprivation Therapy.
4. Patients who are on 5-alpha reductase inhibitors such as Proscar. If they were on such therapy and discontinued at least 12 months prior to randomization, then they are eligible.
5. Patients with KPS less than 80%.
6. Patients with co-morbidities that lead to life expectancy of less than 5 years.
7. Patients who are unable to sign an informed consent.
8. Patients with other simultaneous or second malignancies within 5 years of registration.
9. Patients who had prostatectomies as part of treatment for prostate cancer or other conditions [for example, Abdomino-Perineal resection for rectal cancer].
10. PSA at registration exceeding a value of 10 ng/ml or less than 2 ng/ml.
11. Patients who are considering fathering children.
12. Patients who are unable to swallow and retain oral medicine.
13. Patients who would require a consent form that has to be translated into another language (i.e., a language other than English).
14. Patients with existing hypercalcemia.
15. Patients with existing hypercalcicuria.
16. Patients with existing hyperparathyroidism.
17. Patients with existing sarcoidosis.
18. Patients with existing type distal renal tubular acidosis (type 1 RTA).
19. Patients with existing osteoporosis.
20. Patients with existing renal insufficiency (creatinine clearance <60mL/min/1.72m^2, based on the Cockcroft-Gault equation which allows the creatinine clearance to be estimated from the plasma creatinine in a patient with a stable plasma creatinine.)

$$\text{CCr, in mL/min} = \frac{(140 - \text{age}) \times \text{lean body weight [kg]}}{\text{PCr [mg/dL]} \times 72}$$

21. Patients with a history of hypercalcemia while using vitamin D or vitamin D analogs.
22. Patients with a history of calcium-containing kidney stones.
23. Patients with a history of hypercalemia-related pancreatitis.

3. RISKS

INFORMED CONSENT PROCESS

Potential subjects will be patients from the clinics of the study investigators. The investigators will make the initial contact and will assess the inclusion/exclusion criteria for potential subjects using interviews. The discussion that the investigators will have with potential subjects will closely follow the text of the consent form (see attachment). The patient and his family will be given a consent form to take home and read, and will be encouraged to write down their questions. During the next patient visit approximately one week later, the consent form will be discussed further, and the potential subjects will be asked to confirm that they have read the description of the study or have had it translated into a language that they understand. They also will be able to discuss the study with their doctors until all questions are answered. Potential subjects will be asked to state that they understand: (1) that the study is to determine whether or not the treatment is effective and tolerated, rather than how effective it is; (2) that their participation is voluntary; and (3) that they know enough about the purpose, methods, risks, and benefits of the study to judge that they want to participate. Each potential subject must be able to provide informed consent, which will be obtained by the investigators.

Risks: Use of Specimens

There are very few risks to subjects. The greatest risk is the release of information from their health records, which may be necessary for investigators to obtain along with their specimens. Investigators will protect subjects' records so that their name, address, and phone number will be kept private.

Potential Risks and Discomforts

There are a number of potential risks and discomforts that subjects will be made aware of before they consent to participate. Subjects will be informed of any significant new findings developed during the course of the research that could affect their willingness to continue participation. The investigational agent to be used in this study is not approved by the Food and Drug Administration (FDA) for commercial use; however, FDA has permitted its use in this research study.

Potential Side Effects and Complications from the Study Medications

Although preliminary studies have indicated a relative safety of the Study Medication, one of the purposes of this study is to see whether there are any unexpected side effects from it. One of the

known side effects of Vitamin D, when taken in excess or when the potent analogs are used, is an increase in blood calcium levels – this is called "Hypercalcemia". The symptoms of Hypercalcemia are listed in the tables below.

Some Potential Risks Associated with the Study Procedures		
Procedures	***Risks***	***Measures to Minimize Risks***
Taking the drug 1α(OH)D5 for 2 years	Hypercalcemia: symptoms include loss of appetite, nausea, vomiting, abdominal pain, constipation, and other symptoms (see 2nd table below). There may also be unknown effects since the study drug is a newly synthesized analog of vitamin D.	During the 2-year treatment period, subjects will be examined weekly, monthly, and then every 4 months. They also will be called by phone by the Clinical Research Associate weekly or monthly about the side effects they are experiencing, and the dosage of study drug will be adjusted or will be stopped temporarily or permanently as necessary.
Providing blood samples weekly, monthly, and every four months (2-3 tbsp each), over the course of two years	1) Pain, local bruising, bleeding, possible infection. 2) Possible breach of confidentiality.	1) Blood collection methods used in the study are the same as those used for routine clinical exams. 2) Procedures have been established for confidential collection, labeling, storage, use, and disposal of blood samples.
Ultrasound guided biopsy of prostate at the end of two years	Since needle biopsy will be used, risks are discomfort, local bleeding, small bruise, tenderness, infection (rare), and allergic reaction to local anesthesia.	
Completing "Quality of Life" survey several times during 2-year period	Inconvenience of completing forms	
Telephone interviews by Clinical Research Associate done weekly or monthly during 2-year period	Inconvenience of completing interviews	
Physical exam and digital rectal exam by radiation oncologist several times during 2-year period	Minor discomfort	

Symptoms of Hypercalcemia can be:

- Loss of appetite, nausea, vomiting, abdominal pain, constipation, inflammation of pancreas, stomach or intestinal ulcers
- Confusion, memory loss, tiredness, depression, even fainting
- Excessive urination, more frequent urination, including at night, kidney stone formation
- Muscle weakness, muscle aches, bone pain
- Increase in blood pressure, calcium deposits in the soft tissues of the body, a band formation in the cornea of the eye
- Itching
- HOWEVER, MOST PATIENTS DO NOT HAVE ANY SYMPTOMS. THAT IS ONE OF THE REASONS WE HAVE DESIGNED THIS STUDY WITH A PERIOD OF INTENSIVE FOLLOW-UP IN THE INITIAL FOUR MONTHS: TO IDENTIFY ANY OF THESE SYMPTOMS EARLY AND INTERVENE IF NECESSARY.
- ALSO, DEVELOPING SYMPTOMS DEPENDS UPON HOW LONG AND HOW RAPIDLY CALCIUM LEVELS INCREASE IN THE BLOOD. THE SHORTER THE DURATION AND LESS RAPID THE INCREASE, THE LESS ARE THE CHANCES OF DEVELOPING SIDE EFFECTS. THAT IS WHY, AGAIN, WE HAVE DESIGNED THE INTENSIVE FOLLOW-UP PERIOD TO DETECT ANY HYPERCALCEMIA AS SOON AS POSSIBLE, IF IT OCCURS.

- There may be other unknown and unexpected complications that could occur, including life-threatening complications.

Blood Drawing

The most frequent risks are bruising, pain at the site of needle stick, bleeding, and infection. The amount of blood drawn is unlikely to lead to anemia (low blood cell count).

Follow-up visits and completion of forms

Generally, prostate cancer patients are seen every four months after they complete radiotherapy, undergo a doctor's examination (including a digital rectal examination), and get blood drawn at the time of follow-up visits for PSA. So the follow-up schedule for the study is not any different than in other patients except during the initial phases. In addition, the number of telephone calls and the necessity of completing many forms can be inconvenient and may interfere with subjects' routine life.

Biopsy

This has the same risks and discomforts as the biopsy subjects had at the time of their diagnosis: A needle biopsy can be painful. Risks include bleeding and infection. Subjects may notice blood in their urine, in their semen, or with a bowel movement for several weeks after the biopsy.

What if a subject is injured as a result of participation?
All forms of medical diagnosis, treatment, and research, whether routine or experimental, involve some risk of injury. In spite of all precautions, subjects might develop complications from participation in this study.

If subjects are hurt or get sick because of this research study, they can receive medical care at an Army hospital or clinic free of charge. They will only be treated for injuries that are directly caused by the research study. The Army will not pay for subjects' transportation to and from the hospital or clinic. If subjects have questions about this medical care, they should talk to the principal investigator for this study, Dr. Srinivasan Vijayakumar, at (916) 734-7888. If subjects pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221.

Subjects may, if they wish, receive treatment for a research-related injury at the UCD Medical Center. There is no compensation and/or payment for such medical treatment from the UCD Medical Center for such injury except as may be required of the University by law.

Should subjects feel they have been injured, they may contact:

- Dr. Vijayakumar, Principal Investigator, at (916) 734-7888
- Dr. Narayan at (916) 734-8051
- Dr. Ryu at (916) 734-8251
- Any of our Clinical Research Associates:
    - Clinical Research Nurse (to be named)
    - Cheri Grelle at (916) 734-3604
    - Cathy Hollister at (916) 734-8814

All routine diagnostic laboratory tests and follow-up office visit costs necessary for subjects' treatment will be borne by their insurance company (i.e., HMO or other health benefit provider). However, if their insurance company refuses to reimburse them, then subjects will be billed for these procedures. There will be no charge for the drug(s) or some of the specific tests performed to gather scientific information regarding this form of vitamin D. The biopsy at the end of the study carries the same risks as the biopsy subjects had at the time of diagnosis, and will not cost them any additional expense.

ADVERSE EVENT REPORTING

An adverse event is defined as any untoward medical occurrence that occurs from the first dose of study medication until 30 days after the final dose, regardless of whether it is considered related to a medication. In addition, any known untoward event that occurs subsequent to the

adverse event reporting period that the investigator assesses as possibly related to the investigational medication should also be considered an adverse event.

A serious adverse event is one that is fatal or life-threatening (i.e., results in an immediate risk of death), is permanently or substantially disabling, requires or prolongs hospitalization (only if related to an unexpected complication), is a new cancer or a medication overdose. This category also includes any other event the investigator judges to be serious or which would suggest a significant hazard, contraindication, side effect or precaution.

An unexpected event is one that is not listed as a known toxicity of the investigational drug in the protocol or the consent form.

Submission of Adverse Event Reports

Unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study, and all subject deaths will be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to the Army Surgeon General's Human Subjects Research Review Board (HSRRB). A complete written report should follow the initial telephone call. In addition to the methods above, the complete report can be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-QH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

Adverse experiences that are both serious and unexpected will be immediately reported by telephone to the USAMRMC Deputy for Regulatory Compliance and Quality (see above).

The MEDWATCH adverse event reporting form (Appendix III) and this study's own Adverse Event Report Form (Appendix IV) will be used to report adverse events.

Because this study is being conducted under an Investigator IND, serious unexpected adverse events must be reported to the FDA and to the IRB within 10 working days. The Department of Defense, as the study sponsor, will be provided with a copy of all adverse events filed with the FDA.

The address for submitting serious adverse event reports to the FDA is:

MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Phone: (301) 230-2330
FAX #: 1-800-FDA-0178

A copy of the submitted report will also be sent to the Principal Investigator, Dr. Srinivasan Vijayakumar, by fax (916) 734-7076 or by e-mail (vijay@ucdavis.edu) for distribution to all participating study physicians, nurses and coordinators. The Adverse Event Report Form (Appendix IV) should be sent to the Principal Investigator within 24 hours. Any supporting

documentation (i.e., laboratory, pathology, progress notes, discharge summary, autopsy, etc.) explaining the AE should accompany the submitted report.

Questions regarding adverse event reporting should be directed to the Clinical Research Associates, Cheri Grelle (916-734-3604, beeper 916-762-1601) or Cathy Hollister (916-734-8814, beeper 916-762-6282).

MEDICAL MONITOR REQUIREMENT

Per HSRRB requirements, a medical monitor is assigned to this study. The name and *curriculum vitae* of the medical monitor is provided. This individual is a qualified physician who is not associated with this particular protocol, is able to provide medical care to research subjects for conditions that may arise during the conduct of the study, and will monitor the subjects during the conduct of the study. The medical monitor is required to review all serious and unexpected adverse events (per ICH definitions) associated with the protocol and provide an unbiased written report of the event within 10 calendar days of the initial report. At a minimum, the medical monitor will comment on the outcomes of the adverse event (AE) and relationship of the AE to the test article. The medical monitor will also indicate whether he/she concurs with the details of the report provided by the study investigator.

The medical monitor for this study is Dr. Allan Chen of U.C. Davis Cancer Center.

PROCEDURES FOR MAINTAINING AND BREAKING RANDOMIZATION CODES

The treatment randomization codes will be devised and maintained by our department programmer, Alan Wu, Ph.D. Dr. Wu has no other responsibilities or involvement in the trial. Using a computer program, he will randomly assign patients in the study to either the study drug or placebo group. He will then share this information with study personnel at the University of Illinois at Chicago (UIC), who will prepare coded drug bottles for each study subject. UIC will ship the coded drug bottles to the UC Davis Pharmacy at the Cancer Center. There, UC Davis Clinical Research Associates will obtain the coded drug bottles from the UC Davis Pharmacy and dispense them to study subjects, according to the protocol. Thus, only Dr. Wu and UIC personnel will be unblinded as to which subjects are receiving the study drug.

Should an adverse event occur, the study's Principal Investigator will inform the Medical Monitor (as well as other appropriate entities). The Medical Monitor will ask Dr. Wu which study group (treatment or placebo) the study patient was in. If Dr. Wu is not available, s/he will contact study personnel at UIC for that information. The Medical Monitor will then undertake the actions described under the "Medical Monitor Requirement" section of this protocol and make appropriate recommendations to the study's Principal Investigator.

MONITORING OF SIDE EFFECTS DURING ONE-MONTH RUN-IN PERIOD

During the one-month run-in period, when study subjects are taking a placebo to judge their ability to comply with pill-taking requirements of the study, any side effects or adverse events will be monitored by the Clinical Research Associates. Since the study subjects will be taking a

placebo, no side effects are anticipated. However, the study subjects will be given the phone numbers of all the relevant study personnel, including the Principal Investigator, other study physicians, and the Clinical Research Associates.

MODIFICATION OF PROTOCOL

The Principal investigator does not expect that the protocol will be modified and terminated, or extended. However, should there be a need for one of these to occur, the Principal Investigator will make such changes only with UCD IRB approval, the consent of the Cancer Center Data Monitoring Committee, and the Department of Defense. Any protocol modification is to be reviewed and approved by the HSRRB of the DOD prior to implementation of the modification. Similarly, HSRRB will be notified of any deviations from the protocol.

INTERVENTIONS DURING FOLLOW-UP PERIOD

The expected course of events is:

- I. Completely asymptomatic or with minimal symptoms as expected after RT. → NO INTERVENTION; CONTINUE STUDY
- II. Severe Symptoms related to RT [for example: rectal ulceration, hematuria]. [These events are unusual with an expected rate of less than 1%.] → INTERVENTION FOR THE TREATMENT OF THE CONDITION; DRUG HOLIDAY; RESUME MEDICATION AFTER PATIENT RECOVERS
- III. Increasing PSA:
  - If 3 consecutive increases and Dt < 12 months →
    - Off Study.
    - Immediate Biopsy
- IV. Clinical Evidence of Local Recurrence →
  - Biopsy
    - \- → Continue on Study
    - \+ →
      - Off Study.
      - Treat per standard clinical practice
  - Continue follow-up.
- V. Evidence of Metastatic Disease: →
- VI. Reportable Study Medication-related complications: (Grade 3 and above) →

There is no rescue medication for this study. Study subjects experiencing adverse effects from the study medication (D5) will stop taking D5 and be provided necessary clinical support.

Most research-related injuries will be treated and resolved by the research institution, UC Davis Medical Center, which will follow its own policy for emergency care, as related in the informed consent form to the subject. In the event of a subject's needing non-emergency care, the PI will call the Army if the PI has a subject with a research-related injury that the PI's institution is unwilling to treat, or if the subject for some reason wants to explore Army treatment (at an Army Medical Treatment Facility) even though the institution has offered treatment.

The PI will be able to tell the subject where the nearest Army MTF is by looking at this website for a list: http://www.armymedicine.army.mil/default2.htm (click on Leaders and Organizations, then under Organizations, click on US Army Medical Department Organization Chart, then click on See All Online Army Medical Facilities). The PI cannot promise medical care from that Army MTF as the PI is not the one who will be making determination of eligibility. The PI will inform the study subject that if the Army finds him eligible for Army MTF care (because the Army agrees that the injury is research-related), then it is possible that subject can get medical care at an Army MTF. However, the subject should not call the Army MTF directly, because that is not how eligibility will be determined.

4. BENEFITS

Subjects may receive no direct benefit for participation in this study. Their participation will help other patients if Vitamin D5 is found to be an effective drug in preventing prostate cancer recurrence. As subjects will be randomized to treatment and control groups, ~50% of participants will receive D5. Those subjects would directly benefit from the hypothetical reduction in prostate cancer recurrence resulting from D5. Thus this research with Vitamin D5 might help people who have prostate cancer and other cancers in the future. The benefits of this research include improved understanding of prostate cancer treatment, recurrence prevention, and prophylaxis.

5. RISK-BENEFIT RATIO

This study poses minimal risk to participants and large potential benefit to future prostate cancer patients. Vitamin D5 has been shown to be safe and tolerable in animal models using doses in excess of several times the proposed dose used in this study. Furthermore, participants will be strictly monitored and followed for the development of any side effects or adverse reactions due to the administration of D5. It is our opinion that Vitamin D5 is a safe medication and is highly unlikely to result in significant side effects or adverse reactions. Vitamin D5 has also demonstrated anti-tumor activity against prostate cancer cell lines using both in vitro as well as in vivo animal models. It is our hypothesis that this effect will translate into reduction in the recurrence of prostate cancer in individuals who are at high risk to recur. No treatment modalities for the prevention of prostate cancer recurrence are currently available. Vitamin D5 may represent significant preventive treatment and ultimately provide direct benefit, measurable in reduced recurrence rates, in the ~50% of participants randomized to receive D5 treatment. As the theoretical risks to the administration of vitamin D5 are low and adequate steps have been

undertaken to recognize and manage these risks, it is our opinion that the treatment arm is at low risk in this study. The placebo arm, by nature of the study design is at even lower risk of side effects or complications. It is also our hypothesis that vitamin D5 will provide direct benefits to those patients randomized to the treatment arm. If D5 is effective in preventing prostate cancer recurrence the large potential benefit to future prostate cancer patients would be immeasurable. It is therefore our opinion that the benefits of undertaking this study of vitamin D5 far outweigh the risks.

6. COSTS TO SUBJECTS

Subjects will not be charged or paid to participate in the study. The study medications will be provided to subjects free of cost. The routine blood tests that are part of their regular follow-up will be paid by either the insurance company or by the patient, as in the case of a patient who had received radiotherapy and was being followed by his doctors. Subjects will not be charged for any of the particular blood tests that are specifically designed for the study.

It is possible that their insurance will not pay for all of the treatments and tests subjects will receive if they participate in the research. That is because many insurance companies, HMOs, and health benefits plans do not cover experimental treatments. Subjects will give us permission to submit bills to any appropriate third parties (insurance carriers).

All routine diagnostic laboratory tests and follow-up office visit costs necessary for subjects' treatment will be borne by their insurance company (i.e., HMO or other health benefit provider). However, if their insurance company refuses to reimburse subjects, then they will be billed for these procedures. There will be no charge for the drug(s) or some of the specific tests performed to gather scientific information regarding this form of vitamin D. The biopsy at the end of the study carries the same risks as the biopsy subjects had at the time of diagnosis, and will not be charged to the patient. This biopsy is not part of the patient's standard care as a prostate cancer patient who has had radiation therapy.

As stated above, if subjects are hurt or get sick because of this research study, they can receive medical care at an Army hospital or clinic free of charge. Subjects will only be treated for injuries that are directly caused by the research study. The Army will not pay for participants' transportation to and from the hospital or clinic.

7. DISCLOSURE OF PERSONAL AND FINANCIAL INTEREST IN THE RESEARCH STUDY AND SPONSOR

The principal investigator, co-investigators and sponsoring agency, the Department of Defense, have no personal or financial interests in this research study.

8. RESOURCES

The Department of Defense (DOD) has given the principal investigator a grant to conduct this study. The detailed budget given to the DOD shows that adequate funds have been allotted for

personnel (% of time for principal investigator, co-investigators, research nurse, statistician), consultants, travel, subject-related costs, and other expenses.

Srinivasan Vijayakumar, M.D. Dr. Vijayakumar serves as the PI for this project. He is responsible for the overall project. Specifically, he is responsible for the clinical protocol, which will include all aspects of the radiation therapy and treatment with vitamin D5, follow-up, and pathology as well as clinical chemistry. Dr. Vijayakumar will spend 5% of his time on the project.

Ralph deVere White, M.D. Dr. deVere White will serve as Urologist on the project, assisting Dr. Vijayakumar with the clinical studies and obtaining biopsies. Dr. de Vere White will spend 2% of his time on the project.

Research Nurse (TBN). A nurse will assist Dr. Vijayakumar with the clinical studies. S/he will spend 25% of her/his time on the project.

Laurel Beckett, Ph.D., Statistician. Dr. Beckett will assist with the experimental design, sample size, and statistical analyses. She will be used on an as-needed basis with an effort commitment of 1% to 1.5% per year.

The following co-invesitigators will spend less than 1% of their time on the project:

Ralph Green, M.D., Pathologist. Dr. Green will collaborate on the project for the purposes of identification of PIN and other pathological conditions.

Samir Narayan, M.D.; Janice Ryu, M.D.; William Baker, M.D. These co-investigators will enroll patients into the clinical trial.

Paul Gumerlock, M.D. Dr. Gumerlock will assist with this study as it relates to the genetics of prostate cancer.

Rajendra Mehta, Ph.D. and Dr. Rajeshwari Mehta, Ph.D. These two co-investigators will conduct preliminary studies with D5, share their expertise in developing the appropriate doses of D5 for humans, and analyze data from the project.

Alan Diamond, Ph.D. Dr. Diamond will provide nutritional advice to the project, as needed.

Cathy Hollister and Cheri Grelle, Clinical Research Associates. Ms. Hollister and Ms. Grelle will assist with coordination of the project, as needed (the Clinical Research Nurse will have primary responsibility for this).

In addition, investigators have the invaluable resource of the U.C. Davis Cancer Center, where the study is being conducted. There is no cost to study participants. There is no compensation for participating in the study.

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ABBREVIATIONS

$1\alpha(OH)D_5$ = 1α-Hydroxyvitamin D5, 1αhydroxy-24-ethyl-cholecalciferol, A vitamin D analog synthesized at the University of Illinois at Chicago

AdEERS = Adverse Event Expedited Reporting System
AE = Adverse Event
AI = Adequate Intake
ASTRO = American Society for Therapeutic Radiology
CRA = Clinical Research Associate
CTC = Common Toxicity Criteria
CTCAE = Common Terminology Criteria for Adverse Events
DCT = Division of Cancer Therapy
DOD = Department of Defense
DRE = Digital Rectal Examination
DU-145 = Prostate cancer cell line
FDA = Food and Drug Administration
GCP = Good Clinical Practice
GLP = Good Laboratory Practice
GMP = Good Manufacturing Practice
HSRRB = Human Subjects Research Review Board (of DOD)
ICH = the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.
IDB = Investigational Drug Branch
IU = International Units
LNCaP = Prostate cancer cell line
Mcg = micrograms
MNU = Methyl nitrosourea
MTD = Maximum Tolerated Dose
NIH = National Institutes of Health
PC-3 = Prostate cancer cell line
PSMA = Prostate-Specific Membrane Antigen
PSA = Prostate-Specific Antigen
QOL = Quality of Life
RDA = Recommended Dietary Allowance
RT = Radiation Therapy
TBN = To Be Named
TGF = Transforming growth factor
UCD = University of California, Davis
UCDMC = University of California, Davis Medical Center
UL = Upper Intake Level
USAMRMC = U.S. Army Medical Research and Materiel Command
UV = ultraviolet
VDR = Vitamin D receptor
VDRE = Vitamin D response element

APPENDIX I

KARNOFSKY PERFORMANCE SCALE

Patient I.D. Sticker:

SCORE	DESCRIPTION
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self, unable to carry on normal activity or do active work
60	Requires occasional assistance, but is able to care for most of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled, requires special care and assistance
30	Severely disabled, hospitalization indicated Death not imminent
20	Very sick, hospitalization indicated. Death not imminent
10	Moribund, fatal processes progressing rapidly
0	Death

APPENDIX II

STAGING CRITERIA

Patient I.D. Sticker:

DEFINITIONS
Tumor (T), Node (N), Metastases (M)
Classification Prostate Cancer

Primary Tumor (T)

TX		Primary tumor cannot be assessed
T0		No evidence of primary tumor
T1		Clinically inapparent tumor not palpable or visible by imaging
	T1a	Tumor incidental histologic finding in 5 % or less of tissue resected
	T1b	Tumor incidental histologic finding in more than 5 % of tissue resected
	T1c	Tumor identified by needle biopsy (e.g., because of elevated PSA)
T2		Palpable tumor confined within prostate*
	T2a	Tumor involves half of a lobe
	T2b	Tumor involves more than half of a lobe, but not both lobes
	T2c	Tumor involves both lobes
T3		Tumor extends through the prostatic capsule **
	T3a	Unilateral extracapsular extension
	T3b	Bilateral extracapsular extension
	T3c	Tumor invades seminal vesicle
T4		Tumor is fixed or invades adjacent structures other than seminal vesicles
	T4a	Tumor external sphincter and/or bladder neck and /or rectum
	T4b	Tumor invades levator muscles and/or is fixed to pelvic wall

Lymph Node (N)

NX	Regional lymph nodes cannot be assessed
N0	No regional node metastasis
N1	Metastasis in a single lymph node, 2 cm or less in greatest dimension
N2	Metastasis in a single lymph node, more than 2 cm but not more than 5 cm greatest dimension or multiple lymph nodes, none more than 5 cm in greatest dimension
N3	Metastasis in a lymph node more than 5 cm in greatest dimension

Distant Metastasis (M) ***

MX	Presence of distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

M1a Non-regional lymph nodes
M2b Bone
M3c Other sites

---

* Note: Tumor found in one or both lobes by needle biopsy, but not palpable or visible by imaging is classified as T1c

** **Note: Invasion into the prostatic apex or into (but not beyond) the prostatic capsule is not classified as T3, but as T2**

*** **Note: When more than one site of metastasis is present, the most advanced category (M1c) is used.**

U.S. Department of Health and Human Services

MEDWATCH

The FDA Safety Information and Adverse Event Reporting Program

For VOLUNTARY reporting of adverse events and product problems

Page ____ of ____

Form Approved: OMB No. 0910-0291, Expires: 03/31/05
See OMB statement on reverse.

FDA USE ONLY
Triage unit sequence #

PLEASE TYPE OR USE BLACK INK

A. PATIENT INFORMATION

1. Patient Identifier
In confidence

2. Age at Time of Event:
or
Date of Birth:

3. Sex
☐ Female ☐ Male

4. Weight
____ lbs
or
____ kgs

B. ADVERSE EVENT OR PRODUCT PROBLEM

1. ☐ Adverse Event and/or ☐ Product Problem *(e.g., defects/malfunctions)*

2. Outcomes Attributed to Adverse Event *(Check all that apply)*
☐ Death: _____ *(mo/day/yr)*
☐ Life-threatening
☐ Hospitalization - initial or prolonged
☐ Disability
☐ Congenital Anomaly
☐ Required Intervention to Prevent Permanent Impairment/Damage
☐ Other: _____

3. Date of Event *(mo/day/year)*

4. Date of This Report *(mo/day/year)*

5. Describe Event or Problem

6. Relevant Tests/Laboratory Data, Including Dates

7. Other Relevant History, Including Preexisting Medical Conditions *(e.g., allergies, race, pregnancy, smoking and alcohol use, hepatic/renal dysfunction, etc.)*

C. SUSPECT MEDICATION(S)

1. Name *(Give labeled strength & mfr/labeler, if known)*
#1
#2

2. Dose, Frequency & Route Used
#1
#2

3. Therapy Dates *(If unknown, give duration)* from/to (or best estimate)
#1
#2

4. Diagnosis for Use *(Indication)*
#1
#2

5. Event Abated After Use Stopped or Dose Reduced?
#1 ☐ Yes ☐ No ☐ Doesn't Apply
#2 ☐ Yes ☐ No ☐ Doesn't Apply

6. Lot # *(if known)*
#1
#2

7. Exp. Date *(if known)*
#1
#2

8. Event Reappeared After Reintroduction?
#1 ☐ Yes ☐ No ☐ Doesn't Apply
#2 ☐ Yes ☐ No ☐ Doesn't Apply

9. NDC# *(For product problems only)*
– –

10. Concomitant Medical Products and Therapy Dates *(Exclude treatment of event)*

D. SUSPECT MEDICAL DEVICE

1. Brand Name

2. Type of Device

3. Manufacturer Name, City and State

4. Model #
Lot #
Catalog #
Expiration Date *(mo/day/yr)*
Serial #
Other #

5. Operator of Device
☐ Health Professional
☐ Lay User/Patient
☐ Other: _____

6. If Implanted, Give Date *(mo/day/yr)*

7. If Explanted, Give Date *(mo/day/yr)*

8. Is this a Single-use Device that was Reprocessed and Reused on a Patient?
☐ Yes ☐ No

9. If Yes to Item No. 8, Enter Name and Address of Reprocessor

10. Device Available for Evaluation? *(Do not send to FDA)*
☐ Yes ☐ No ☐ Returned to Manufacturer on: _____ *(mo/day/yr)*

11. Concomitant Medical Products and Therapy Dates *(Exclude treatment of event)*

E. REPORTER *(See confidentiality section on back)*

1. Name and Address

Phone #

2. Health Professional?
☐ Yes ☐ No

3. Occupation

4. Also Reported to:
☐ Manufacturer
☐ User Facility
☐ Distributor/Importer

5. If you do NOT want your identity disclosed to the manufacturer, place an "X" in this box: ☐

Mail to: MEDWATCH 5600 Fishers Lane Rockville, MD 20852-9787 -or- FAX to: 1-800-FDA-0178

FORM FDA 3500 (9/03) Submission of a report does not constitute an admission that medical personnel or the product caused or contributed to the event.

APPENDIX IV
ADVERSE EVENT REPORT FORM

A Phase I/II Double-Blinded, Randomized Clinical Trial to Prevent/Delay Biochemical and Clinical Failure in High-Risk, Non-Metastatic Prostate Cancer Patients After Radiotherapy, Using 1α-Hydroxyvitamin D5 Versus Placebo: A Tolerance-Finding and Intermediate Biomarker Response-Seeking Study (HSRRB Log Number: A-11241)

Most frequently expected adverse events for this study:
Hypercalcemia:

- Loss of appetite, nausea, vomiting, abdominal pain, constipation, inflammation of pancreas, stomach or intestinal ulcers
- Confusion, memory loss, tiredness, depression, even fainting
- Excessive urination, more frequent urination, including at night, kidney stone formation
- Muscle weakness, muscle aches, bone pain
- Increase in blood pressure, calcium deposits in the soft tissues of the body, a band formation in the cornea of the eye
- Itching

---

1) Participant I.D. No: _____ 2) Protocol No: _____

3) Participant Initials: _____ 4) Investigator: Srinivasan Vijayakumar, MD

5) Institution Name: U.C. Davis Medical Center

6) Person Completing Form: _____
(Name & Signature)

7) Telephone: _____ Role in Study: _____

8) Randomization Date: _____

9) Study Drug ID number: _____

10) Still taking study drug?: ☐ Yes ☐ No, Date Discontinued: _____

11) Toxicity (per CTC): _____ 12) Toxicity grade: _____

13) Toxicity Category (choose one): ☐ Known ☐ Unknown ☐ Death

14) Attribution: Event related to study drug?
☐ Definitely ☐ Probably ☐ Possibly ☐ Not Likely ☐ Definitely Not

15) Date of Adverse Event Started: _____

ADVERSE EVENT REPORT FORM (page 2)

16) Date of Adverse Event Ended: _____

17) Toxicity Description: _____

_____

_____

_____

18) Pre-existing Conditions: (describe all that apply): _____

_____

_____

_____

19) Number of subjects enrolled to date: _____

20) Number and type of serious and unexpected adverse events reported previously in the study:

_____

_____

21) Description of the Study (e.g., double or single blind; phase of study the subject is participating in): _____

_____

_____

22) Synopsis of the Event: _____

_____

_____

23) Status of the subject: _____

_____

24) Actions taken in response to this event: _____

_____

_____

25) Resolution of the adverse event (include modifications/changes to protocol): _____

_____

_____

_____ _____

Signature of Investigator Date

APPENDIX V

QUALITY OF LIFE FORM

A Phase I/II Double-Blinded, Randomized Clinical Trial to Prevent/Delay Biochemical and Clinical Failure in High-Risk, Non-Metastatic Prostate Cancer Patients After Radiotherapy, Using 1α-Hydroxyvitamin D5 Versus Placebo: A Tolerance-Finding and Intermediate Biomarker Response-Seeking Study (PI: S. Vijayakumar, MD/ HSRRB Log #A-11241)

Sticker:

HEALTH SURVEY SF-36V

Instructions: Please read each question and fill in the box that best describes your experience.

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Answer every question by marking with an "X" the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

Date of form completed: _____

1. **In general, would you say your health is:**

   ☐ Excellent

   ☐ Very Good

   ☐ Good

   ☐ Fair

   ☐ Poor

(continued on next page)

2. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

(SELECT ONE ANSWER FOR EACH QUESTION)

ACTIVITIES	Yes, limited a lot	Yes, limited a little	No, not limited at all
a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	☐	☐	☐
b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?	☐	☐	☐
c. Lifting or carrying groceries	☐	☐	☐
d. Climbing several flights of stairs?	☐	☐	☐
e. Climbing one flight of stairs?	☐	☐	☐
f. Bending, kneeling, or stooping?	☐	☐	☐
g. Walking more than a mile?	☐	☐	☐
h. Walking several blocks?	☐	☐	☐
i. Walking one block?	☐	☐	☐
j. Bathing or dressing yourself?	☐	☐	☐

3. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	No, none of the time	Yes, a little of the time	Yes, some of the time	Yes, most of the time	Yes, all of the time
a. Cut down the amount of time you spent on work or other activities	☐	☐	☐	☐	☐
b. Accomplished lees than you would like	☐	☐	☐	☐	☐
c. Were limited on the kind of work or other activities	☐	☐	☐	☐	☐
d. Had difficulty performing ht work or other activities (for example, it took extra effort)	☐	☐	☐	☐	☐

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious).

	No, none of the time	Yes, a little of the time	Yes, some of the time	Yes, most of the time	Yes, all of the time
a. Cut down the amount of time you spent on work or other activities	☐	☐	☐	☐	☐
b. Accomplished less than you would like	☐	☐	☐	☐	☐
c. Didn't do work or other activities as carefully as usual	☐	☐	☐	☐	☐

5. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

☐ Not at all ☐ Slightly ☐ Moderately ☐ Quite a bit ☐ Extremely

6. How much bodily pain have you had during the past 4 weeks?

☐ None ☐ Very mild ☐ Mild ☐ Moderate ☐ Severe ☐ Very Severe

7. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and house work)?

☐ Not at all ☐ A little bit ☐ Moderately ☐ Quite a bit ☐ Extremely

8. These questions are about how much you feel and how things have been with you during the past 4 weeks. For each question, please give one answer that comes closest to the way you have been feeling.

How much of the time during the past 4 weeks:	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
a. Did you feel full of pep?	☐	☐	☐	☐	☐	☐
b. Have you been a very nervous person?	☐	☐	☐	☐	☐	☐
c. Have you felt so down in the dumps that nothing could cheer you up?	☐	☐	☐	☐	☐	☐
d. Have you felt calm and peaceful?	☐	☐	☐	☐	☐	☐
e. Did you have a lot of energy?	☐	☐	☐	☐	☐	☐
f. Have you felt downhearted and blue?	☐	☐	☐	☐	☐	☐
g. Did you feel worn out?	☐	☐	☐	☐	☐	☐
h. Have you been a happy person?	☐	☐	☐	☐	☐	☐
i. Did you feel tired?	☐	☐	☐	☐	☐	☐

9. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (Like visiting friends, relatives, etc.)?

☐ All of the time ☐ Most of the time ☐ Some of the time ☐ A little of the time ☐ None of the time

10. Please choose the answer that best describes how true or false each of the following statements is for you.

	Definitely True	Mostly True	Not Sure	Mostly False	Definitely False
a. I seem to get sick a little easier than other people	☐	☐	☐	☐	☐
b. I am as healthy as anybody I know	☐	☐	☐	☐	☐
c. I expect my health to get worse	☐	☐	☐	☐	☐
d. My health is excellent	☐	☐	☐	☐	☐

11. Please mark the appropriate box with an X to indicate how would you rate your own quality of life during the past four weeks

Lowest quality applies to someone completely dependent physically on others, seriously troubled mentally, unaware of surroundings and in a hopeless position.

Highest quality applies to someone physically and mentally independent, communicating well with others, able to do most of the things enjoyed, pulling own weight, with a hopeful yet realistic attitude.

Lowest→ Quality	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10	←Highest Quality

Now, we'd like to ask you some questions about your physical health may have changed.

12. Compared to one year ago, how would you rate your physical health in general now?

☐ Much better ☐ Somewhat better ☐ About the same ☐ Somewhat worse ☐ Much worse

13. Compared to one year ago, how would you rate your emotional problems (such as feeling anxious, depressed, or irritable) now?

☐ Much better ☐ Somewhat better ☐ About the same ☐ Somewhat worse ☐ Much worse

APPENDIX VI: AUA GU SYMPTOM SCORING SCALE

A Phase I/II Double-Blinded, Randomized Clinical Trial to Prevent/Delay Biochemical and Clinical Failure in High-Risk, Non-Metastatic Prostate Cancer Patients After Radiotherapy, Using 1α-Hydroxyvitamin D5 Versus Placebo: A Tolerance-Finding and Intermediate Biomarker Response-Seeking Study (PI: S. Vijayakumar, MD/ HSRRB Log #A-11241)

Patient I.D. Sticker:

Please circle your score below.

1. Over the last month or so, how many times did you most typically get up to urinate from the time you went to bed at night until the time you got up in the morning?
☐ None ☐ 1 time ☐ 2 times ☐ 3 times ☐ 4 times ☐ 5 or more times

2. Over the past month or so, how often have you had a sensation of not emptying your bladder completely after you finished urinating?
☐ None ☐ Less than 1 time in 5 ☐ Less than half of time ☐ About half the time ☐ More than half the time ☐ Almost always

3. Over the past month or so, how often have you had to urinate again less than two hours after you finished urinating?
☐ None ☐ Less than 1 time in 5 ☐ Less than half of time ☐ About half the time ☐ More than half the time ☐ Almost always

4. Over the past month or so, how often have you found that you stopped and started again several times when you urinated?
☐ None ☐ Less than 1 time in 5 ☐ Less than half of time ☐ About half the time ☐ More than half the time ☐ Almost always

5. Over the past month or so, how often have you found it difficult to postpone urination?
☐ None ☐ Less than 1 time in 5 ☐ Less than half of time ☐ About half the time ☐ More than half the time ☐ Almost always

6. Over the past month or so, how often have you had a weak urinary stream?
☐ None ☐ Less than 1 time in 5 ☐ Less than half of time ☐ About half the time ☐ More than half the time ☐ Almost always

7. Over the past month or so, how often have you had to push or strain to begin urination?
☐ None ☐ Less than 1 time in 5 ☐ Less than half of time ☐ About half the time ☐ More than half the time ☐ Almost always

TOTAL SCORE: _____/35

APPENDIX VII

END OF STUDY BIOPSY REPORTING FORM

Patient I.D. Sticker

1. Date of Procedure: ☐☐/☐☐/☐☐

   If procedure was refused, enter date of refusal

2. Prostate Diagram

   Outline the hypoechoic Transrectal Ultrasound findings. Place an X at the location of each biopsy site

3. Ultrasound Probe Characteristics

   MHZ of probe ☐☐☐.☐

4. Ultrasound Sizing: All measurements should be made to obtain maximum dimension

   Prostate Size
   a. Widths (axial plane): ☐☐.☐ cm
   b. Antero-posterior: ☐☐.☐ cm
   c. Length (longitudinal): ☐☐.☐ cm

(continued on next page)

5. Summary of Findings:

Region #	Echogenicity			DRE Results		Biopsied?		Extension through capsule		Seminal vesicle invasion	
	hypo	iso	hyper	nml	abnl	yes	no	yes	no	yes	no
1	☐	☐	☐	☐	☐	☐	☐	☐	☐		
2	☐	☐	☐	☐	☐	☐	☐	☐	☐		
3	☐	☐	☐	☐	☐	☐	☐	☐	☐		
4	☐	☐	☐	☐	☐	☐	☐	☐	☐		
5	☐	☐	☐	☐	☐	☐	☐	☐	☐		
6	☐	☐	☐	☐	☐	☐	☐	☐	☐		
7	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
8	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
9	☐	☐	☐	☐	☐	☐	☐	☐	☐		
10	☐	☐	☐	☐	☐	☐	☐	☐	☐		
11	☐	☐	☐	☐	☐	☐	☐	☐	☐		
12	☐	☐	☐	☐	☐	☐	☐	☐	☐		

General Comments:

_____

_____

_____

_____

1. Record results of the DRE done at the time of TRUS with biopsy. If DRE was not done at this time, please indicate in General Comments.

2. Record the seminal vesicle invasion as determined by TRUS only.

PILL DIARY • U.C. DAVIS CANCER CENTER • RADIATION ONCOLOGY DEPT

A Phase I/II Double-Blinded, Randomized Clinical Trial to Prevent/Delay Biochemical and Clinical Failure in High-Risk, Non-Metastatic Prostate Cancer Patients After Radiotherapy, Using 1α-Hydroxyvitamin D5 Versus Placebo: A Tolerance-Finding and Intermediate Biomarker Response-Seeking Study (PI: S. Vijayakumar, MD/ HSRRB Log #A-11241)

Patient's Name: _____

Instructions for the Patient: This is a monthly calendar on which you are to record the number of pills you are taking. Be sure you have enough calendars to last until your next appointment. If you develop any side effects from the pill, mark this on the calendar on the day you note the effect. **Bring the bottle(s) with the unused pills and your calendars with you each time you have an appointment.**

If you have any questions, contact: _____ Telephone: _____

Your next appointment is: _____

SPECIAL INSTRUCTIONS: _____

_____

MONTH: _____

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY

_____

Patient Signature Date

Section to be completed by the nurse or research associate. Review the pill diary and check for toxicities. Report adverse reactions and toxicities according to protocol instructions. Complete the items below and update the specific Flow Sheet.

Report period: Start date: ___/___/___ (mm/dd/yy) End date: ___/___/___ (mm/dd/yy) Total pills taken this month_____ based on pill count

COMMENTS: _____

_____

Signature: _____ Date: _____

APPENDIX IX

TISSUE SAMPLE CONSENT FORM
CONSENT FOR USE OF SPECIMENS FOR
FUTURE RESEARCH PURPOSES
(tissue, blood, urine and other body materials)

CONSENT TO USE OF SPECIMENS FOR RESEARCH
UNIVERSITY OF CALIFORNIA, DAVIS

page 1 of 4

Investigator's Name(s): Srinivasan Vijayakumar, M.D., Ralph W. deVere White, M.D, Samir Narayan, M.D., Janice K. Ryu, M.D., Paul Gumerlock, M.D., Laurel Beckett, Ph.D., Ralph Green, M.D.

Department: Radiation Oncology, Urology, Hematology & Oncology, Epidemiology & Preventive Medicine, Pathology

Telephone Number(s): (916) 734-7888, (916) 734-3604 Emergency Phone: (916) 734-2011

Using Specimens for Research Purposes

At the time of your surgery or biopsy, a small piece of tissue was removed for diagnosis. We would like to keep some of the tissue that is left for **future** research purposes. If you agree, these **specimen(s)** will be kept and used to learn more about your disease as well as other diseases.

The research that may be done with your specimen(s) probably will not benefit you directly nor have an effect on your care, nor will it prevent you from participating in other research. It might help people who have your disease and other diseases in the future. Any reports about the research, done with your specimen(s), will not be shared with you or your doctor and the reports will not be put in your health record. No identifying information such as your name, address or phone number will be indicated in any research report.

Things to Think About

The decision to let us keep the specimen(s) for research purposes is up to you. No matter what you decide to do, it will not affect your care.

Even if you have already consented to let us use your specimen(s), you can change your mind at any time. Just let us know that you do not want us to use your specimen(s) and it will no longer be used.

Sometimes tissue/blood/urine are used for genetic research (about diseases that are passed on in families). Even if your tissue or blood is used for this kind of research, the results will not be put in your health records.

Your specimen(s) will only be used for research purposes. The research done with your specimen(s) may help to develop new products in the future. Please be aware that you will not have any property rights or ownership interests in products or data which may be derived from the use of your specimen(s).

Benefits

The benefits of research using specimens include learning more about what causes diseases, how to prevent them, how to treat them, and how to cure them.

Risks

There are very few risks to you. The greatest risk is the release of information from your health records which may be necessary for us to obtain along with your specimens. We will protect your records so that your name, address, and phone number will be kept private.

Where Do Specimens Come From?

Generally, a specimen may be from a blood sample, urine, or from bone marrow, skin, toenails or other body materials (in this study, the biopsy specimen will come from your prostate). People who are trained to handle specimens and protect donors' rights make sure that the highest standards of quality control are followed.

Why Do People Do Research With Specimens?

Research with specimens can help to find out more about diseases, how to prevent them, how to treat them, and how to cure them.

What Type of Research Will Be Done With My Specimen?

Many different kinds of studies use specimens. Some researchers may develop new tests to find diseases. Others may develop new ways to treat and even cure diseases. In the future, some of the research may help to develop new products, such as tests and drugs. Some research looks at diseases that are passed on in families (called genetic research). Research done with your specimen may look for genetic causes and signs of disease.

page 3 of 4

Will I Find Out the Results of the Research Using My Specimen?

You will not receive the results of research done with your specimen. This is because research can take a long time and must use specimen samples from many people before results are known. Results from research using your specimen may not be ready for many years and will not affect your care right now, but they may be helpful to people like you in the future.

Why Do You Need Information From My Health Records?

In order to do research with your specimen, researchers may need to know some things about you. (For example: are you male or female? What is your race or ethnic group? How old are you? Have you ever smoked?) This helps researchers answer questions about diseases. The information that will be given to the researcher may include your age, sex, race, diagnosis, treatments, and family history. This information is collected by your hospital from your health record.

Will My Name Be Attached to the Records That Are Given to the Researcher?

No, you will remain anonymous. Your sample will be identified by a case number, which can be linked to your personal information (your name, disease classifications, ethnic status, family history), which will be kept in a secure data bank with our statistician.

How could the Records Be Used in Ways That Might Be Harmful To Me?

Sometimes, health records have been used against patients and their families. For example, insurance companies may deny a patient insurance or employers may not hire someone with a certain illness (such as AIDS or cancer). The results of genetic research may not apply only to you, but to your family members too. For disease caused by gene changes, the information in one person's health record could be used against family members.

How Am I Protected?

Your name, address, phone number and any other identifying information will be taken off anything associated with your specimen before it is given to the researcher. Your tissue will be stored by case number. This case number can be linked to your personal information, which is kept in a secure Data Bank with our Clinical Research Associates.

What If I Have More Questions?

If you have any questions, please talk to the research investigator who provided you this form.

page 4 of 4

CONSENT

Your signature below will indicate that you will allow us to use your specimens(s) for future research purposes. You will be given a signed and dated copy of this form to keep.

Signature of Donor _____ **Date** _____

Signature of Principal Investigator _____ **Date** _____

APPENDIX X

DOD Vitamin D5
UC Davis – Department of Radiation Oncology
ELIGIBILITY CRITERIA - CHECKLIST

Subject Initials: _____

Subject Number: _____

☐ Subject has completed radiotherapy with curative intent within 5 years from the date of registration, but not within the immediate twelve months.

Radiotherapy could have been external beam RT [XRT] alone, XRT with neoadjuvant hormonal therapy of brief duration [not exceeding 12 months], brachytherapy alone, brachytherapy with neoadjuvant hormonal therapy of brief duration [not exceeding 12 months], or a combination of XRT and brachytherapy [again, if neoadjuvant hormonal therapy was given, it should have been for a duration not exceeding 12 months]

☐ Subject had Digital Rectal Examination and documentation of the pre-RT findings in a AJCC Staging Sheet.

☐ Subject had Pre-treatment biopsy with pathology report of Gleason Sum.

☐ Subject had documented non-metastatic prostate cancer, i.e., no clinical or imaging evidence of distant metastases or lymph-node metastases.

☐ Pre treatment PSA level is between 2 and 8

☐ PSA has been stable [no more than 0.75 ng/ml variation in the PSA value], with at least 3 measurements within 12 months prior to the date of registration. (PSA doubling time must be ≤ 6 months)

☐ Subject is classified as Group II or III based on T-stage, Gleason Sum and PSA criteria:

(not eligible) Group I = T1/T2 AND Gleason Sum <6 AND PSA < 10 ng/ml

☐ Group II = One of the three factors higher than under Group I

☐ Group III = Two or more of the three factors higher than under Group I

☐ Subject has no evidence of metastatic disease at the time of registration.

☐ Subject is not currently on Androgen Deprivation Therapy.

☐ Subject is not currently on and has not used 5-α reductase inhibitor, such as Proscar, within the last 12 months.

☐ Subject Karnofsky Performance Status [KPS] is ≥80%.

☐ Subject has no simultaneous or second malignancies within 5 years of registration.

☐ Subject did not undergo prostatectomy as part of treatment for prostate cancer or other conditions

☐ Subject has signed and been given a copy of the informed consent form.

☐ Subject is ≥18 years of age. (There is no maximum age limit for study subjects.)

☐ Subject has no future plans to father children.

☐ Subject is able to swallow and retain oral medicine.

Study Coordinator_____ Date_____

DOD Vitamin D5 Initial Visit Form
University of California Davis
Department of Radiation Oncology

Subject Initials _____

Subject Number _____

Instructions: Complete this form at the appropriate follow-up visit and whenever there is a change in the patient's status.
Use-0 for unknown or not applicable unless otherwise specified in the code table.

1 ___/___/____ Date of Assessment

2 ☐ Quality of Life Form Complete
1 Not Completed
2 Completed

3 ☐ AUA GU Symptom Scale Complete
1 Not Completed
2 Completed

4 ☐☐☐ Karnosfky Performance Status
(9= unknown)

5 ☐ Digital Rectal Examination
0. Not Done/Unknown
1. No Palpable Disease
2. Palpable Disease

6 ☐ + ☐ = ☐ Pre-treatment Gleason Score

7 Pre-Treatment TNM Stage

T	N	M

8 Baseline Laboratory Values
1 Not Done
2 Normal
3 Abnormal
4 Unknown

☐ Chemistry Panel
Date ___/___/_____

Na	Cl	BUN	Glu
K	HCO3	Cr	

☐ Calcium, Magnesium, Phosphate
Date ___/___/_____

Ca	Mg	PO4

☐ Albumin
Date ___/___/_____

Alb

☐ PTH
Date ___/___/_____

PTH

☐ Urine Electrolytes
Date ___/___/_____

uNa	uK	uCl	uCr	uCa

9 Current Medications

1 _____

2 _____

3 _____

4 _____

5 _____

6 _____

7 _____

8 _____

9 _____

10 _____

11 _____

12 _____

10 Additional Treatments Since Completion of Radation Therapy

☐ 1 No (Skip to end of form)
2 Yes (Complete form)
9 Unknown

☐ Additional Therapy For Prostate Cancer or Complications of Initial Prostate Cancer Treatment
Specify _____

_____

☐ Additional Therapy For Prostate or Other Genitourinary Conditions/Treatments
Specify _____

_____

☐ Additional Medications or Therapies (For Any Condition) Since Last Follow-up Visit
Specify _____

_____

_____

_____

11 Comments _____

_____

_____

_____

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_____

_____
Signature

_____
Date

DOD Vitamin D5 Follow-Up Visit Form
University of California Davis
Department of Radiation Oncology

Subject Initials _____

Subject Number _____

Instructions: Complete this form at the appropriate follow-up visit and whenever there is a change in the patient's status.
Use-0 for unknown or not applicable unless otherwise specified in the code table.

1 ___/___/____ Date of Assessment

2 ☐ Quality of Life Form Complete
1 Not Completed
2 Completed

3 ☐ AUA GU Symptom Scale Complete
1 Not Completed
2 Completed

4 ☐☐☐ Karnosfky Performance Status
(9= unknown)

5 ☐ Digital Rectal Examination
0. Not Done/Unknown
1. No Palpable Disease
2. Palpable Disease

6 ☐☐☐ Weight

7 ☐☐☐ Pill Count (Count remaining pills)

8 Baseline Laboratory Values
1 Not Done
2 Normal
3 Abnormal
4 Unknown

☐ Chemistry Panel
Date ___/___/_____

Na	Cl	BUN	Glu
K	HCO3	Cr	

☐ Calcium, Magnesium, Phosphate
Date ___/___/_____

Ca	Mg	PO4

☐ Albumin
Date ___/___/_____

Alb

☐ PTH
Date ___/___/_____

PTH

☐ Urine Electrolytes
Date ___/___/_____

uNa	uK	uCl	uCr	uCa

APPENDIX XII

9 Complications of Treatment (Record date of 1st appearance. Use 0=absent and 1=present. If reaction is severe please give a description)

- ☐ Asymptomatic Hypercalcemia ___/___/_____
- ☐ Hot Flashes ___/___/_____
- ☐ Nausea ___/___/_____
- ☐ Emesis ___/___/_____
- ☐ Diarrhea ___/___/_____
- ☐ Abdominal Pain ___/___/_____
- ☐ Loss of Appetite ___/___/_____
- ☐ Renal Calculi ___/___/_____
- ☐ Bone Pain ___/___/_____
- ☐ Other Gastrointestinal ___/___/_____
  Specify_____
- ☐ Other Genitourinary ___/___/_____
  Specify_____
- ☐ Hematologic ___/___/_____
  Specify_____
- ☐ Dermatologic ___/___/_____
  Specify_____
- ☐ Cardiovascular ___/___/_____
  Specify_____
- ☐ Other ___/___/_____
  Specify_____

10 Additional Treatments Since Last Follow-up Visit

☐
1 No (Skip to end of form)
2 Yes (Complete form)
9 Unknown

☐ Additional Therapy For Prostate Cancer or Complications of Initial Prostate Cancer Treatment
Specify_____
_____

☐ Additional Therapy For Prostate or Other Genitourinary Conditions/Treatments
Specify_____
_____

☐ Additional Medications or Therapies (For Any Condition) Since Last Follow-up Visit
Specify_____
_____
_____
_____

11 Comments_____
_____
_____
_____
_____
_____

_____
Signature

_____
Date

DOD Vitamin D5 Telephone Contact Form
University of California Davis
Department of Radiation Oncology

Subject Initials _____

Subject Number _____

Instructions: Complete this form at the appropriate follow-up visit and whenever there is a change in the patient's status.
Use-0 for unknown or not applicable unless otherwise specified in the code table.

1 Complications of Treatment (Record date of 1st appearance. Use 0=absent and 1=present. If reaction is severe please give a description)

- ☐ Asymptomatic Hypercalcemia ___/___/_____
- ☐ Hot Flashes ___/___/_____
- ☐ Nausea ___/___/_____
- ☐ Emesis ___/___/_____
- ☐ Diarrhea ___/___/_____
- ☐ Abdominal Pain ___/___/_____
- ☐ Loss of Appetite ___/___/_____
- ☐ Renal Calculi ___/___/_____
- ☐ Bone Pain ___/___/_____
- ☐ Other Gastrointestinal ___/___/_____
  Specify_____
- ☐ Other Genitourinary ___/___/_____
  Specify_____
- ☐ Hematologic ___/___/_____
  Specify_____
- ☐ Dermatologic ___/___/_____
  Specify_____
- ☐ Cardiovascular ___/___/_____
  Specify_____
- ☐ Other ___/___/_____
  Specify_____

2 Additional Treatments Since Last Follow-up Visit

☐
1 No (Skip to end of form)
2 Yes (Complete form)
9 Unknown

☐ Additional Therapy For Prostate Cancer or Complications of Initial Prostate Cancer Treatment
Specify_____

☐ Additional Therapy For Prostate or Other Genitourinary Conditions/Treatments
Specify_____

☐ Additional Medications or Therapies (For Any Condition) Since Last Follow-up Visit
Specify_____

3 Comments_____

_____
Signature

_____
Date

DOD Vitamin D5 Study Completion Form
University of California Davis
Department of Radiation Oncology

Subject Initials _____

Subject Number _____

Instructions: Complete this form at the appropriate follow-up visit and whenever there is a change in the patient's status.
Use-0 for unknown or not applicable unless otherwise specified in the code table.

1 ___/___/____ Date of Assessment

2 ☐ Quality of Life Form Complete
1 Not Completed
2 Completed

3 ☐ AUA GU Symptom Scale Complete
1 Not Completed
2 Completed

4 ☐☐☐ Karnosfky Performance Status
(9= unknown)

5 ☐ Digital Rectal Examination
0. Not Done/Unknown
1. No Palpable Disease
2. Palpable Disease

6 ☐☐☐ Weight

7 ☐☐☐ Pill Count (Count remaining pills)
** Please collect all Study Medications

8 Final Laboratory Values
1 Not Done
2 Normal
3 Abnormal
4 Unknown

☐ Chemistry Panel
Date ___/___/_____

Na	Cl	BUN	Glu
K	HCO3	Cr	

☐ Calcium, Magnesium, Phosphate
Date ___/___/_____

Ca	Mg	PO4

☐ Albumin
Date ___/___/_____

Alb

☐ PTH
Date ___/___/_____

PTH

☐ Urine Electrolytes
Date ___/___/_____

uNa	uK	uCl	uCr	uCa

9 Complications of Treatment (Record date of 1st appearance. Use 0=absent and 1=present. If reaction is severe please give a description)

- ☐ Asymptomatic Hypercalcemia ___/___/_____
- ☐ Hot Flashes ___/___/_____
- ☐ Nausea ___/___/_____
- ☐ Emesis ___/___/_____
- ☐ Diarrhea ___/___/_____
- ☐ Abdominal Pain ___/___/_____
- ☐ Loss of Appetite ___/___/_____
- ☐ Renal Calculi ___/___/_____
- ☐ Bone Pain ___/___/_____
- ☐ Other Gastrointestinal ___/___/_____
  Specify_____
- ☐ Other Genitourinary ___/___/_____
  Specify_____
- ☐ Hematologic ___/___/_____
  Specify_____
- ☐ Dermatologic ___/___/_____
  Specify_____
- ☐ Cardiovascular ___/___/_____
  Specify_____
- ☐ Other ___/___/_____
  Specify_____

10 Additional Treatments Since Last Follow-up Visit

☐
1 No (Skip to end of form)
2 Yes (Complete form)
9 Unknown

☐ Additional Therapy For Prostate Cancer or Complications of Initial Prostate Cancer Treatment
Specify_____
_____

☐ Additional Therapy For Prostate or Other Genitourinary Conditions/Treatments
Specify_____
_____

☐ Additional Medications or Therapies (For Any Condition) Since Last Follow-up Visit
Specify_____
_____
_____
_____

12 End of Study Biopsy Completed

☐
1 No
2 Yes
9 Unknown

Biopsy Findings _____
_____
_____
_____
_____
_____
_____

11 Comments_____

_____

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_____
Signature

_____
Date

UNIVERSITY OF CALIFORNIA DAVIS
MEDICAL CENTER,
SACRAMENTO, CALIFORNIA

CONSENT TO OPERATION, PROCEDURES, BLOOD TRANSFUSION AND ANESTHESIA

The purpose of this form is to advise you of important information regarding the operation or procedure(s) that your doctor has recommended to you. **PLEASE READ THE ENTIRE FORM CAREFULLY BEFORE SIGNING IT.**

I authorize ______________________, M.D., and those who he/she may designate as associates or assistants to perform the following operation or medical procedure ____

____

____ as well as any related or incidental diagnostic or therapeutic procedures that they believe may be necessary.

I understand that I will be informed of any substitution of the doctor named above and will be given the opportunity to refuse substitution.

I ACKNOWLEDGE THAT THE FOLLOWING INFORMATION HAS BEEN EXPLAINED TO ME:
(a) The purpose and expected benefits of the proposed operation or procedure described above;
(b) significant risks or possible complications that are known to be associated with it;
(c) reasonable alternative methods of treatment (if any);
(d) The possible effects to my health if I should refuse to undergo the operation or procedure; and
(e) research or economic interests (if any) that are related to the performance of this operation or procedure.

BLOOD TRANSFUSIONS

I understand that unless a medical emergency exists, or it was determined to be medically inadvisable, my doctor will have informed me if there was a reasonable possibility that a transfusion or blood or blood components may be necessary. I understand that there are various options available to me regarding blood transfusion, including the right to refuse blood or blood components. I understand that refusing transfusions that are recommended by my doctor(s) may result in life-threatening consequences to me.

I understand that certain risks and complications may be associated with blood transfusions, including, but not limited to transmission of infectious diseases and transfusion reaction.

ANESTHESIA

I authorize the administration of anesthesia if it is determined to be necessary to assure my safety and comfort. I understand that certain risks and complications may be associated with anesthesia use and that they have been discussed with me, as well as reasonable alternative choices of anesthesia (if any).

AUTHORIZATION AND CONSENT:

By my signature below, I confirm that:
(1) I have read this form; ______________________
(2) I have been given the opportunity to discuss with my doctor(s) any questions that I may have regarding the nature and purpose of this operation or procedure, and my questions have been answered fully and to my satisfaction;
(3) I understand that the operation or procedure may not accomplish the desired purpose and that no promises or guarantees of any kind have been made to me as to the result or cure; and
(4) I understand that extra services such as laboratory studies or x-rays may be ordered if determined by my doctor(s) to be necessary;
(5) I have the right to consent or to refuse any proposed operation or procedure prior to its performance.

PATHOLOGY SERVICES

I authorize the hospital pathologist, at his or her discretion, to retain, preserve, or dispose of any tissues, organs or medical devices that may be removed during the procedure subject to the following conditions (if any),

NO INFORMATION REQUESTED:

Although given the opportunity to have this information explained to me, I specifically decline to be advised of the nature, benefit, risks and alternatives to the proposed operation/procedure as well as those associated w/anesthesia.

______________________ ____
Date PATIENT OR PATIENT'S LEGAL REPRESENTATIVE AND RELATIONSHIP OF REP. TO THE PATIENT

______________________ ____
Time INFORMANT AND PRINTED NAME OF INFORMANT

Curriculum Vitae

ALLAN YI-NAN CHEN, M.D., Ph.D.

Assistant Professor in Residence
Department of Radiation Oncology
UC Davis Cancer Center
4501 X Street, G-126
Sacramento, CA 95817
Work: 916-734-8252
FAX: 916-454-4614
e-mail: allan.chen@ucdmc.ucdavis.edu

EDUCATION

Taipei Medical College, Taipei, Taiwan	Doctor of Medicine	1978 – 1985
Johns Hopkins Medical School, Baltimore, Maryland	Ph.D. in Biochemistry Cellular and Molecular Biology	1990 – 1993
Transitional Program, Fairfax Hospital/ Georgetown University, Falls Church, Virginia	Internship	1/94 – 12/94
Radiation Oncology Branch National Cancer Institute National Institutes of Health, Bethesda Maryland	Residency	7/95 –7/98

EXPERIENCE

Research Assistant, Institute of Molecular Biology, Academic Sinica, Nankang, Taipei, Taiwan	6/87 – 12/87
Visiting Fellow, Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD	1/88 – 12/89
Postdoctoral Fellow, Department of Biological Chemistry, Johns Hopkins Medical School, Baltimore, MD	2/90 – 8/90
Postdoctoral Fellow, Department of Pharmacology, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ	1/93 – 12/93
Biotechnology Fellow, Radiation Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD	1/95 – 6/95

AWARDED RESEARCH SUPPORT

National Research and Science Association Scholarship, Biochemistry, Cellular and Molecular Biology Program, Johns Hopkins Medical School, Baltimore, MD	1990-1991
Leukemia Society Special Fellowship Award, Mechanism of Action of DNA Minor Groove-binding Drugs, Department of Pharmacology, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ	7/93 – 12/94

Basic Science Travel Grant, ASTRO 38th Annual Meeting, Los Angeles, CA	1996
Roentgen Resident/Fellow Research Award, Radiological Society of North America (RSNA)	1997
Accepted as a life member of the National Registry of Who's Who 2002 edition. Registration Number: 185-232	2001
UCDHS Capital Research Equipment Funding RS 2000 Biological Irradiator	5/2002
Faculty Research Grant, Committee on Research, UC Davis Academic Senate Enhancement of Radiotherapy with DNA Topoisomerase I-targeted DB-67 in Human Breast Cancer Xenograft in Nude Mice Model	7/2002 - 6/2003
Research Grant, Univ. of California Cancer Research Coordinating Committee DNA Topoisomerase I-mediated radiation sensitization	7/2002 – 6/2003
Kentucky Lung Cancer Research Program (co-investigator; 5% effort without salary) Anti-Topoisomerase I Aerosols for Lung Cancer Therapy	2002 - present
A Phase I/II Study of Irinotecan and Whole Brain Radiation Therapy in Patients with Brain Metastases from Solid Tumors. Sponsored by Pharmacia & Upjohn.	2002– 12/2004
UCDHS Research Award Program, DNA Topoisomerase I-mediated radiation sensitization	7/2003 – 6/2005
American Cancer Society Institutional Research Grant #IRG-95-125-07, 8th Cycle Chemoradiation with DNA Topoisomerase I-targeted DB-67 in Human Glioma Xenograft in Nude Mice Model	8/2003 – 7/2004
Vanderbilt Award #02-0111SV, Molecular Analysis Services (business contract)	1/2003-11/2004

CERTIFICATION

Board Certified in Radiation Oncology, American Board of Radiology	1999
Tennessee Medical License	1998-2001
Maryland Medical License	1995-1998
California Medical License	2001-present
IMRT School at Emory University (20 hour course)	4/2002
Leksell Gamma Knife Certified users	2003

ACADEMIC APPOINTMENTS

Adjunct Assistant Professor, Department of Pharmacology, University of Medical and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ	4/94 – 1996
Assistant Professor and Director of Drug Discovery Laboratory, Department of Radiation Oncology, Vanderbilt University Medical Center, Nashville, TN	8/98 – 6/2001
Assistant Professor, Dept. of Radiation Oncology, UC Davis Med. Ctr.,	8/01 – present

Sacramento, CA

Director of Radiosurgery Program in UCD, UC Davis Med. Ctr., Sacramento, CA 12/01 – present

MEMBERSHIPS

Society of Chinese Bioscientists in America	1990-present
American Association of Cancer Research	1993-1996; 1999-present
American Society of Therapeutic Radiology and Oncology	1995-present
American College of Radiation Oncology	1996-present
American Society of Clinical Oncology	1999-present
Southwest Oncology Group (SWOG) a Nation Clinical Research Group	2002-present
Sierra Sacramento Valley Medical Society (SSVMS)	2/2004-present
California Medical Association (CMA)	2/2004-present

Exam Committee:

Chau Phan	Ph.D. Thesis Committee	(Pending Advancement)

PROFESSIONAL SERVICE

School of Medicine

Member, Ad Hoc Committee	1/04-present
Member, UC Davis, Graduate Group in Pharmacology & Toxicology	2/02 – present
Member, Research Affairs Committee	6/04-present

UC Davis Cancer Center

Member, Quality Assurance Committee	6/03-present
Member, Cancer Committee	7/04-present

Department of Radiation Oncology

Member, Quality Assurance Committee	8/01-present

RESEARCH INTERESTS

1. Mechanism of Chemoradiation of DNA Topoisomerase I and II Drugs.
2. Development of Novel Radiation Sensitizers.
3. Stereotactic Radiosurgery for CNS Disorders.

ARTICLES

1 1989 Hwang J, Shyy S, **Chen AY**, Juan CC and Whang-Peng J. Studies of topoisomerase-specific antitumor drugs in human lymphocytes using rabbit antisera against recombinant human Topoisomerase 11 polypeptide. *Cancer Research* 49:958-962.

2 1989 Bates SE, Mickley LA, **Chen Y-N**, Richert N, Rudick J, Biedler JL and Fojo AT. Expression of a drug resistance gene in human neuroblastoma cell lines: modulation by retinoic acid-induced differentiation. *Mol. Cell Biol.* 9:4337-4344.

3 1990 **Chen Y-N,** Mickley LA, Schwartz AM, Acton EM, Hwang J and Fojo AT. Characterization of Adriamycin-resistant human breast cancer cells which display overexpression of a novel resistance-related membrane protein. *J. Biol. Chem.* 265(17):10073-10080.

4 1991 Lai GM, **Chen A-Y**, Mickley LA, Fojo AT and Bates SE. P-glycoprotein expression and schedule dependence of adriamycin cytotoxicity in human colon carcinoma cell lines. *Int. J. Cancer* 49:696-703.

5 1991 **Chen AY,** Yu C, Potmesil M, Wall ME, Wani MC and Liu LF. Camptothecin overcomes MDR1-mediated resistance in human KB carcinoma cells. *Cancer Res.* 51:6039-6044.

6 1993 **Chen AY,** Yu C, Bodley A, Peng LF and Liu FL. A new mammalian DNA topoisomerase I poison hoechst 33342: cytotoxicity and drug resistance in human cell cultures. *Cancer Res.* 53:1332-1337.

7 1993 **Chen AY,** Yu C, Gatto B and Liu LF. DNA minor groove-binding ligands: a different class of mammalian DNA topoisomerase I inhibitors. *Proc. Natl. Acad. Sci.* 90:8131-8135.

8 1993 Luo Y, Ren Y-F, Chou T-C, **Chen AY**, Yu C, Liu LF and Cheng CC. A structure-activity relationship study of batracylin analogues. *Pharm. Res.* 10(6):918-923.

9 1994 **Chen AY** and Liu LF. DNA topoisomerases: Essential Enzymes & Lethal Targets. *Annu. Rev. Pharmacol. Toxicol.* 34:191-218.

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12 1994 Meegalla SK, Stevens GJ, McQueen CA, **Chen AY**, Yu C, Liu LF, LR Barrows and LaVoie EJ. Synthesis & Pharmacological Evaluation of Isoindolo[1,2-b]quinazolinone & Isoindolo[2,1-a]benzimidazole Derivatives Related to the Antitumor Agent Batracylin. *J. Med. Chem.* 37:3434-3439.

13 1997 Abraham EH, Okunieff P, Scala S, Vos P, Oosterveld M, **Chen AY**, Shrivastav B and Guidotti G. Cystic fibrosis transmembrane conductance regulator and adenosine triphosphate. *Science* 275:1324-1326.

14 1997 **Chen AY,** Okunieff P, Pommier Y and Mitchell JB. Mammalian DNA topoisomerase I mediates the enhancement of radiation cytotoxicity by camptothecin derivatives. *Cancer Res.* 57:1529-36.

15 1999 Li T-K, **Chen AY**, Yu C, Mao Y, Wang H and Liu LF. Activation of topoisomerase II-mediated excision of chromosomal DNA loops during oxidative stress. *Genes & Dev* 13:1553-1560.

16 1999 **Chen AY,** Choy H and Rothenberg ML. DNA topoisomerase I-targeting drugs as radiation sensitizers. *Oncology* 13 (10):39-46.

17 1999 Hallahan DE, **Chen AY**, Teng M and Cmelak AJ. Drug-radiation interactions in tumor blood vessels. *Oncology* 13 (10):71-77.

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20 2004 **Chen AY,** Chou R, Shih S-J, Lau D and Gandara D. Enhancement of Radiotherapy with DNA Topoisomerase I-targeted Drugs. *Crit Rev Oncol Hematol* 50:111-119.

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22 2004 **Chen AY,** Shih L, Hsiao M, Rothenberg ML and Prudhomme M. Induction of DNA topoisomerase I-mediated radiosensitization by indolocarbazole derivatives. *Molecular Pharmacology* (in press).

23 2004 **Chen, A. Y.**, Phan, C., Chang, Y.-C. & Shih, S.-J. Targeted Radiosensitization with DNA Topoisomerase I Drugs. Discovery Medicine (In press)

ARTICLES SUBMITTED

1 2004 **Chen AY,** Shih S-J, Garriques L, Hsiao M, Rothenberg M, Burke TG and Curran DP. Silatecan DB-67 Induces DNA Topoisomerase I-mediated Radiosensitization in Human Glioma Cells. Submitted to *Int. J. Radiat. Oncol. Biol. Phys.*

2 2004 Shih S-J, Erbele T and **Chen AY**: Ku86 Modulates Camptothecin-induced Radiosensitization in Mammalian Cells. Submitted to *Cancer Research.*

3 2004 Perks J, Yang C, Hartman J, Sahrahkar K, Pappas C and **Chen AY**. Linear Accelerator Based Radiosurgery in a Patient with Four Arterio-venous Malformations. Submitted to *Am J Neuroradiol.*

4 2004 Chou RH, **Chen AY** and Lau D. Promising Role of Irinotecan for the Treatment of Brain Metastases. Submitted to *J Neurooncology.*

5 2004 Perks J, **Chen AY**, Kubo HD, Stern R, El-Hamri K and Plowman PN. Considerations in the Optimal Radiation Therapy Management of Acoustic Neuroma. Submitted to *Proc. 6th Internatl. Stereotactic Radiosurgery Soc. Cong.*

MANUSCRIPTS IN PREPARATION

1 Shih, S.-J., Erbele, T. Phan, C & **Chen, A. Y.** DNA-PK modulates camptothecin-induced radiosensitization in mammalian cells.

2 Shih, S.-J., Garriques, L., & **Chen, A. Y.** Cellular Determinants for DNA Topoisomerase I-mediated Radiosensitization in Mammalian Cells.

3 **Chen, A. Y.**, Yu, C., Cheng, C. C. & Liu, L. F. ATP-independent DNA Topoisomerase II-targeting Batracylin Derivatives Overcome Multidrug Resistance Mechanisms.

BOOK CHAPTERS

1 1992 **Chen AY,** Yu C, Cheng CC, Potmesil M, Wall ME, Wani MC and Liu LF. Topoisomerase poisons that overcome MDR1-mediated resistance, in "Molecular Biology of DNA

Topoisomerases & Its Application to Chemotherapy," Andoh T, Ikeda H and Oguro M(eds.), CRC Press, Boca Raton, Florida, pp. 247-254.

2 1994 **Chen AY** and Liu LF. Design of Topoisomerase Inhibitors to Overcome MDR1-Mediated Drug Resistance in Human Cancers, in "DNA Topoisomerases," Liu LF (eds.), MA, pp. 245-256.

3 1994 **Chen AY** and Liu LF. Mechanisms of Resistance to Topoisomerase Inhibitors, in "Anticancer Drug Resistance: Advances in Molecular and Clinical Research," L. Goldstein and R.F. Ozols (eds.), Kluwer Academic Press, Massachusetts, pp. 263-281.

ABSTRACTS

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2. 1990 **Chen Y-N,** Liu LC, Mickley LA, Brawley O, Hamilton TH, Reed E and Fojo AT. Cisplatin Resistance in Human Ovarian Carcinoma Cell Lines. Proc. Am. Assoc. Cancer Res., 31:1983.

3. 1992 Hendricks CB, **Chen AY,** Yu C, Bodley A and Liu LF. Menogaril Induces Topoisomerase II-mediated DNA Cleavage. Proc. Am. Assoc. Cancer Res., 33: 2587.

4. 1992 **Chen AY,** Yu C, Lee W-H, Peng LF and Liu LF. Menadione (vit. K3) Induces Topoisomerase II-mediated DNA Cleavage. Proc. Am. Assoc. Cancer Res., 33: 2588.

5. 1992 Burke TG, Malak H, Doroshow J, **Chen AY** and Liu L. Model Membrane Interactions of Camptothecin Probed Using Fluorescence Technique. Proc. Am. Assoc. Cancer Res., 33: 2602.

6. 1993 **Chen AY,** Liu LF, Constin D, Wall ME, Wani MC, Silber R and Potmesil M. 10,11-Methylenedioxy Derivatives: Biochemistry of Second-generation Camptothecin Analogues. Proc. Am. Assoc. Cancer Res., 34: 1945.

7. 1994 **Chen AY,** Yu C, Gatto B and Liu LF. Induction of Mammalian DNA Topoisomerase I-mediated DNA Cleavage by the Antitumor Anthracycline Nogalamycin. *Proc. Am. Assoc. Cancer Res.*, 35: 2723.

8. 1994 LaVoie EJ, Sun Q, **Chen AY,** Yu C, Gatto B and Liu LF. Structure-activity Studies Related to Minor Groove-binding Ligands which Inhibit Mammalian DNA Topoisomerase I. *Proc. Am. Assoc. Cancer Res.*, 33:2699.

9. 1999 **Chen AY** and Scruggs PB. Enhancement of Radiation Cytotoxicity of Mammalian Cells by DNA topoisomerase II Drugs. *Proc. Am. Assoc. Cancer Res.*, 40:4220.

10. 2000 **Chen AY,** Scruggs PB and Hallahan DE. Cellular Determinants for DNA Topoisomerase I-mediated Radiosensitization in Mammalian Cells. *Proc. Am. Assoc. Cancer Res.*, 41:1897.

11. 2000 **Chen AY,** Scruggs PB, Riou JF, Prudhomme M and Hallahan D. DNA Topoisomerase I (TOP1)-Targeted Indolocarbazole (INDO) Derivatives Enhance Radiation Cytotoxicity in Mammalian Cells. *Int. J. Radiat. Oncol. Biol. Phys.*, 48 (3S):162-63.

12. 2001 **Chen AY,** Hsiao M, Li L, Rothenberg M, Burke TG and Curran DP. Silatecan DB-67 Induces DNA Topoisomerase I-mediated Radiosensitization in Human Glioma Cells. *Proc. Am. Assoc. Cancer Res.*, 42: 4678.

13. 2002 **Chen AY,** Erbele T and Shih S-J. Suppression of DNA topoisomerase I-mediated radiosensitization in Ku80-deficient mammalian cells. *Proc. Am. Assoc. Cancer Res.*, 43: 5727.

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15. 2003 Shih S-J, Erbele T, Garriques L and **Chen AY.** Ku80 affects camptothecin induced DNA topoisomerase I-mediated radiosensitization. *Proc. Am. Assoc. Cancer Res.*, 44.

16. 2003 Garriques LN, Shih S-J, Erbele T, Singh H and **Chen AY**. p53 and p21 differentially modulate radiation sensitization induced by DNA topoisomerases I and II poisons. *Proc. Am. Assoc. Cancer Res.*, 44.

17. 2003 Perks J, **Chen AY,** Kubo HD, Stern R, El-Hamri K and Plowman PN. Considerations in the optimal radiation therapy management of acoustic neuroma. *Proc. 6th Internatl. Stereotactic Radiosurgery Soc. Cong.*

18. 2003 Shih S-J, Erbele T and **Chen AY.** Ku86 Modulates Camptothecin-induced Radiosensitization in Mammalian Cells. *The 9th Annual Cancer Res. Symp. of UC Davis Cancer Ctr.*

19. 2003 Singh H, Shih S-J, Garriques LN and **Chen AY**. A Laboratory Correlative Study in Patients with Advanced Cancers treated with Oral Topotecan and Etoposide. *The 9th Annual Cancer Res. Symp. of UC Davis Cancer Ctr.*

20. 2003 **Chen AY**, Garriques LN, Shih S-J, Rothenberg M, Burke TG and Curran DP. Silatecan DB-67 Induces DNA Topoisomerase I-mediated Radiosensitization in Human Glioma Cells. *The 9th Annual Cancer Res. Symp. of UC Davis Cancer Ctr.*

21. 2003 Shih S-J, Phan C, Vijayakumar S and **Chen AY**. Modulation of DNA topoisomerase I (TOP1)-mediated radiosensitization by DNA-dependent Protein Kinase. *Int. J. Radiat. Oncol. Biol. Phys.* 57(2S) 146.

22. 2004 **Chen AY**, Garriques LN, Phan C and Shih S-J. Synergistic enhancement by DNA-PK inhibitors of in vitro cytotoxicity from combination of radiation and DNA topoisomerase I- targeted camptothecin. *Proc. Am. Assoc. Cancer Res.*, 45: 1354.

23. 2004 Shih S-J, Garriques LN, Phan C and **Chen AY**. Synergistic enhancement by Ly294002, a DNA-PK inhibitor, of cytotoxicity from combination of radiation and camptothecin in human glioma cells. *Int. J. Radiat. Oncol. Biol. Phys.*, 58 (2S): 2070.

ACCEPTED ABSTRACTS

SUBMITTED ABSTRACTS

INVITED PRESENTATIONS

1 1989 "Multidrug Resistance: Molecular Biology and Clinical Relevance Symposium" (sponsored by National Cancer Institute), Bethesda, Maryland. "A Novel Resistance-related Membrane Protein is Overexpressed in an Adriamycin-resistant MCF-7 Cell Line," April, 1989.

2 1989 Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan. "Beyond MDR1-mediated Drug Resistance," December, 1989.

3 1993 Georgetown University Medical Center, Washington D.C. "New Advances in DNA Topoisomerase-targeting Anticancer Drugs," August, 1993.

4 1993 Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan. "Mechanism of Action and Resistance of DNA Topoisomerase-targeting Anticancer Drugs," December, 1993.

5 1994 American Association of Cancer Research 85th Annual Meeting, San Francisco, CA. "Induction of Mammalian DNA Topoisomerase I-mediated DNA Cleavage by the Antitumor Anthracycline Nogalamycin," April, 1994.

6 1996 American Society of Therapeutic Radiation Oncology 38th Annual Meeting, Los Angeles, CA. "Mammalian DNA Topoisomerase I Mediates the Enhancement of Radiation Cytotoxicity by Camptothecin Derivatives," October, 1996.

7 1998 Department of Radiation Oncology, M.D. Anderson Cancer Center, Houston, TX. "DNA Topoisomerase-targeting Drugs and Radiation," February, 1998.

8 1998 Department of Radiation Oncology, University of Michigan, Ann Arbor, MI "DNA Topoisomerase-targeting Drugs and Radiation," April, 1998.

9 1998 Division of Hematology/Oncology, Vanderbilt University Medical Center, Nashville, TN. "DNA Topoisomerase-targeting Drugs and Radiation," October, 1998.

10 2000 Department of Pharmacology, Wayne State University Medical School, Detroit, MI. "DNA Topoisomerase I-mediated radiation sensitization in mammalian cells," December 2000.

11 2000 American Society of Therapeutic Radiation Oncology 42nd Annual Meeting, Boston, MA. "DNA Topoisomerase I-targeted Indolocarbazole Derivatives Enhance Radiation Cytotoxicity in Mammalian Cells," October, 2000.

12 2000 Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ. "DNA Topoisomerase I-mediated radiation sensitization in mammalian cells," December, 2000.

13 2001 Department of Radiation Oncology, University of California Davis Medical Center, Sacramento, CA. "DNA Topoisomerase I-mediated radiation sensitization in mammalian cells," January, 2001.

14 2001 The Vanderbilt University Symposium "Irinotecan from Scientific Investigation to Clinical Application" (sponsored by the Pharmacia Oncology), Nashville, TN. "DNA Topoisomerase I-mediated radiation sensitization in mammalian cells," March, 2001.

15. 2001 The 11th Conference on DNA Topoisomerases in Therapy, New York, NY. "Enhancement of Radiation Cytotoxicity by Indolocarbazole Derivatives in Mammalian Cells," October 2001.

16. 2001 The 7th Annual Cancer Research Symposium of UC Davis Cancer Ctr., Sacramento, CA. "Mammalian DNA Topoisomerase I-targeted Drugs As Radiation Sensitizers," October 2001.

17. 2002 Department of Neurological Surgery, UC Davis Medical Center, Sacramento, CA. "New Frontier for Brain Tumor Therapy: Enhancement of Radiation Therapy with DNA Topoisomerase I-targeted Radiation Sensitizers," March 2002.

18. 2002 Lung preceptorship with AstraZeneca, UC Davis Medical Center, Sacramento, CA. "Role of DNA Topoisomerase I-targeted Drugs for Locally Advanced NSCLC," April 2002.

19. 2002 Taipei Medical University Hospital, Taipei, Taiwan. "A New Frontier for Brain Tumor Therapy: Enhancement of Radiation Therapy with DNA Topoisomerase I-targeted Radiation Sensitizers," July 2002.

20. 2002 Institute of Public Health, National Yang-Ming Medical University, Taipei, Taiwan. "Enhancement of Radiotherapy with DNA Topoisomerase I-targeted Radiation Sensitizers," July 2002.

21. 2002 Dept. of Medical Education and Research, Kaohsiung Veterans General Hospital, Taiwan. "A New Frontier for Brain Tumor Therapy: Enhancement of Radiation Therapy with DNA Topoisomerase I-targeted Radiation Sensitizers," July 2002.

22. 2002 Department of Radiation Oncology, Kaohsiung Chung-Gan Memorial Hospital, Taiwan. "Enhancement of Radiotherapy with DNA Topoisomerase I-targeted Radiation Sensitizers," July 2002.

23. 2003 The Prostate Cancer Program, UC Davis Cancer Ctr., Sacramento, CA. "The role of NHEJ in DNA topoisomerase I-mediated radiosensitization", February 2003.

24. 2003 Grand Round, UC Davis Cancer Ctr., Sacramento, CA. "Stereotactic Radiosurgery: Cutting-edge Therapy for Intracranial Lesions", Sept. 2003.

25. 2003 The 9th Annual Cancer Research Symposium of UC Davis Cancer Ctr., Sacramento, CA. "Ku86 Modulates Camptothecin-induced Radiosensitization in Mammalian Cells", October 2003.

26. 2003 45th Annual ASTRO Meeting, Salt Lake city, UT. "Modulation of DNA topoisomerase I (TOP1)-mediated radiosensitization by DNA-dependent Protein Kinase ", October 2003.

27. 2003 The 2003 North California Radiation Therapist Association Annual Meeting, Sacramento, CA. "Stereotactic Radiosurgery: Cutting-edge Therapy for Intracranial Lesions", Nov. 2003.

28. 2004 The Research Bldg. III seminar series, UC Davis Cancer Center, Sacramento, CA. "DNA topoisomerase I-mediated radiosensitization, the role of NHEJ", Jan. 2004.

29. 2004 Medicine Academic Conference seminar series, UC Davis Medical Center, Sacramento, CA. "Radiation Oncology 101", June 14, 2004.

30. 2004 Taipei Medical University-Municipal Wan Fang Hospital, Taipei, Taiwan. "Stereotactic Radiosurgery=cutting-edge therapy for intracranial lesions".

INFORMED CONSENT FORM

Cover Sheet